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Marine Ranching

Proceedings of the Seventeenth
U.S.-Japan Meeting on Aquaculture
Ise, Mie Prefecture, Japan
October 16, 17, and 18, 1988

Ralph S. Svrjcek (editor)

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Ralph S. Svrjcek (editor)
Publications Unit
Northwest and Alaska Fisheries Science Centers

Panel Chairmen:
Conrad Mahnken, United States
Hisashi Kan-no, Japan

Under the U.S.-Japan Cooperative Program
in Natural Resources (UJNR)

May 1991

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PREFACE

The United States and Japanese counterpart panels on aquaculture were formed in 1969 under the United States–Japan Cooperative Program in Natural Resources (UJNR). The panels currently include specialists drawn from the federal departments most concerned with aquaculture. Charged with exploring and developing bilateral cooperation, the panels have focused their efforts on exchanging information related to aquaculture which could be of benefit to both countries.

The UJNR was begun during the Third Cabinet-Level Meeting of the Joint United States–Japan Committee on Trade and Economic Affairs in January 1964. In addition to aquaculture, current subjects in the program include desalination of seawater, toxic microorganisms, air pollution, energy, forage crops, national park management, mycoplasmosis, wind and seismic effects, protein resources, forestry, and several joint panels and committees in marine resources research, development, and utilization.

Accomplishments include increased communication and cooperation among technical specialists; exchanges of information, data, and research findings; annual meetings of the panels, a policy-coordinative body; administrative staff meetings; exchanges of equipment, materials, and samples; several major technical conferences; and beneficial effects on international relations.

Conrad Mahnken - United States
Hisashi Kan-no - Japan

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Efficient Techniques for Producing Masu Salmon Smolt and Improving Adult Returns from Outplantings

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ABSTRACT

The goal of enhancing chum salmon (*Oncorhynchus keta*) levels through the introduction of hatchery reared juveniles was reached in the early 1980's. Thus, scientific study became more focused on accomplishing similar progress with the masu salmon (*Oncorhynchus masou*). This paper discusses critical areas of concern for the development of a comprehensive and effective program to improve this important fishery resource. In addition, significant research achievements are reviewed and a basic framework of techniques for smolt production are outlined specificly to the needs of masu salmon.

Introduction

Masu salmon (*Oncorhynchus masou*) usually spend one, sometimes two, years in freshwater after fry emergence from the redds. Young masu salmon which reach the smolt stage migrate down stream to the sea the following spring (Kubo 1980). Environmental devastations in rivers, such as man-made obstructions to fish migration and decreases in water flow resulting from intensive water utilization projects, have resulted in serious decreases in masu salmon resources (Sano 1964; Kobayashi 1980).

In the propagation of masu salmon in Japan, fry have been released from April to June either unfed or after a short-term feeding lasting from 1 to 3 months. This technique is similar to those used in the propagation of chum (*Oncorhynchus keta*) and pink (*O. gorbuscha*) salmon fry that migrate to the sea in early spring soon after their release (Kobayashi 1980). However, the release at the fry-stage has not been an effective method in areas where the environment has been devastated (Kato 1982). In order to augment the decreased masu salmon stocks in these areas, the production of large numbers of smolts in hatchery facilities was considered to be the most promising method, and thus was chosen for further study.

Smolt Production

Masu salmon smolts are normally released as yearlings (age 1 +) in the spring after a rearing procedure mimicking the growth pattern of fish in the natural state (Mayama et al. 1986). Therefore, new technological developments such as underyearling (age 0 +) smolt production (Konno et al. 1983; Sato et al. 1986) are not necessary to produce them. However, in order to achieve high smoltification rates with this method, masu salmon should be reared using strategies closely regulating their growth. Inhibiting growth at the proper time can prevent the early maturation of young males. Conversely, growth acceleration is used to increase the number of fish reaching the minimum size for smoltification by release time.

Males growing to 70 mm or more in fork length by the end of July become sexually mature (Utoh 1976). Thus, techniques used on males to delay egg and sac fry development, with water temperature control and reduced feeding under low water temperature conditions, are considered to be effective for inhibiting the maturation of male during this period. Throughout the winter, from late November through late March, no growth occurs in the natural state under the low water temperatures which are below 5°C. Pond-reared fish, however, tend to grow a little because the water is slightly warmer, and consequently development of the testis often starts (Mayama et al. 1985a). Therefore, growth control during the winter season is also required to achieve a high smoltification rate.

From the results of experimental releases of smolts and fingerlings, it is clear that growth up to 9 cm or more in fork length before overwintering is necessary for smoltification the next spring (Mayama et al. 1988). The ideal

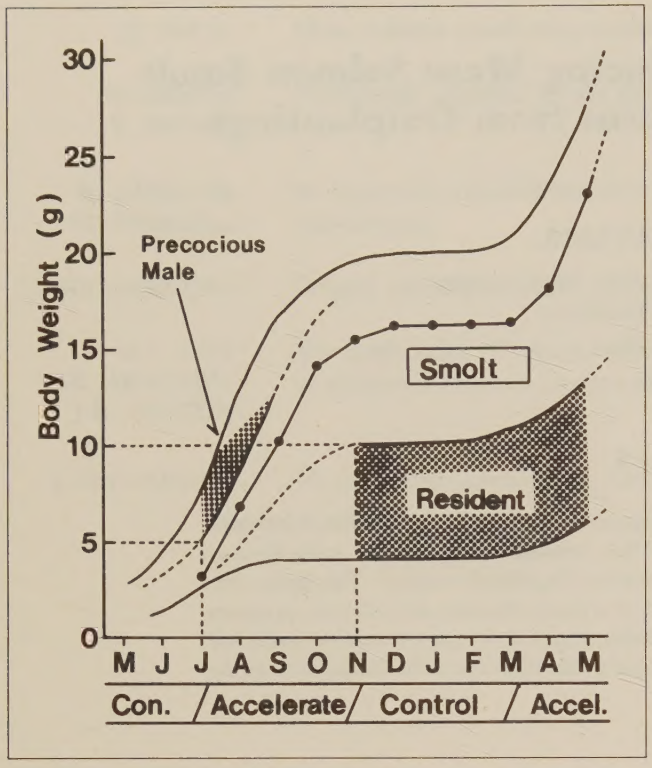

Figure 1

Diagram showing the controlled growth pattern for efficient masu salmon smolt (age 1 +) production in southwestern Hokkaido. Body weight of 5 g in July and 10 g in November are critical points to prevent the early maturation of young males and to reach the minimum size for smoltification by release time, respectively. Ideal growth range (open part between the two critical borderlines) with the average body weight (solid dots) indicates an optimal standard for hatchery-rearing. (Mayama et al. 1986.) M = May; J = June, etc.

growth pattern for efficient smolt production based on the factors described above is shown in Figure 1. The regulated growth curve is divided into four periods:

1. Initial growth control from emergence to early summer (less than 7 cm in fork length, or 5 g in body weight, at a point in late July);
2. Growth acceleration during summer to fall (more than 9 cm in fork length, or 19 g in body weight, by November);
3. Inhibition of growth in the winter season;
4. Growth acceleration prior to smoltification (more than 12 cm in fork length, or 20 g in body weight).

An increase of approximately 20 to 30% in the smoltification rate is obtained by applying this method (Yagisawa and Watanabe 1985; Mayama et al. 1986) resulting in 80% or more conversion to yearling smolts in a large-scale rearing. The diagram applies specifically to masu salmon rearing in south-western Hokkaido. The most effective growth

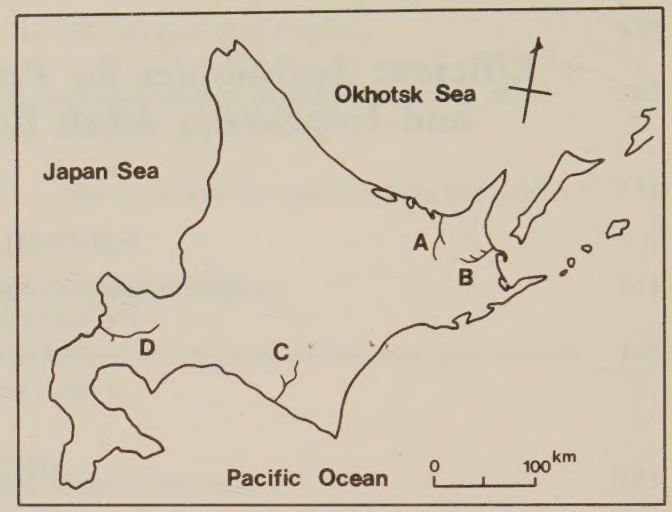

Figure 2

Map showing the locations of the Shari River (**A**), Shibetsu River (**B**), Shizunai River (**C**), and Shiribetsu River (**D**) where experimental smolt releases were carried out.

curve will vary by area because of differences in living condition.

Smolt Release

Most of the technological development studies for releasing masu salmon smolts have been carried out in the Shiribetsu River (Fig. 2) on the Japan Sea coast of Hokkaido since 1981. Much information and knowledge on their migration and growth were obtained from these experiments.

Preliminary Experimental Release

The first experimental smolt release was done in 1981. The results of this experiment were reported previously by Mayama et al. (1985b). The text is summarized here. About 70,000 hatchery produced smolts, averaging 28 g in body weight and 13.8 cm in fork length, were marked by clipping the adipose-fin and released into the Shiribetsu River from late April to early May. About 60,000 fish (except resident parr) migrated to the sea as smolt.

A total of 481 marked fish were recovered in the coastal waters around the home river's mouth from mid-February to late June. The number of marked adults recovered increased in mid-April in accordance with the overall rise in coastal commercial catches.

From the body-weight analysis of the recovered fish it is obvious that the marked salmon were rather small earlier in the study, less than 2 kg until mid-April (Fig. 3). In late April, the average body weight exceeded 2 kg, reaching

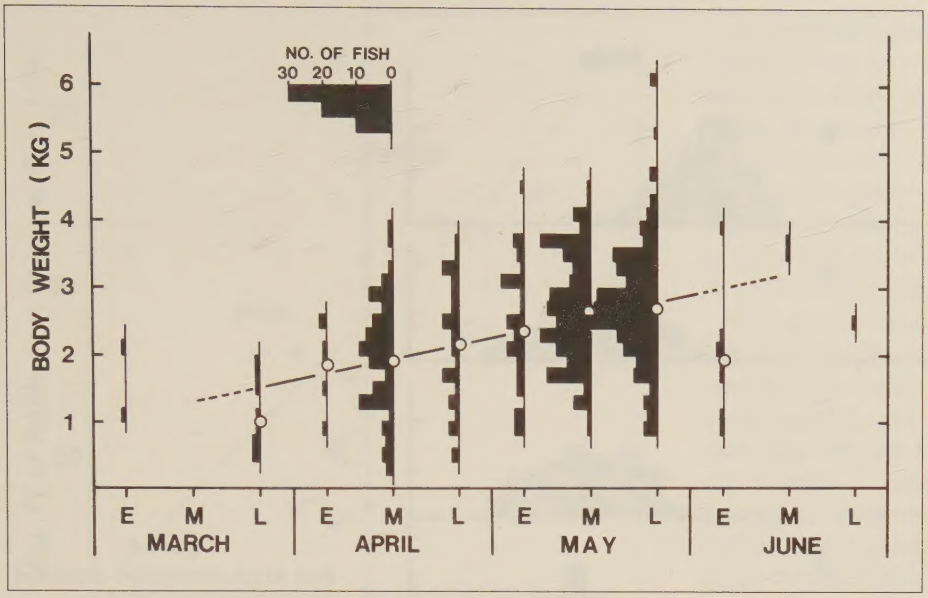

Figure 3
Seasonal change in body weight distribution of marked masu salmon recovered during 10-day periods in coastal waters adjacent to mouth of the Shiribetsu River, 1982. E, M, and L in frame of month indicate early, middle, and late month, respectively. (Mayama et al. 1985b.)

2.7 kg by late May. From this trend of increases it is clear that masu salmon show significant growth from active feeding just before their upstream migration. The fork lengths of marked fish recovered throughout the season in coastal waters ranged from a minimum of 29 cm to a maximum of 70 cm, while body weights varied from 0.2 to 6.1 kg.

Returning adults showed a great variation in body size. The accepted hypothesis that all masu salmon originating in Japan spend one winter in the ocean had been doubted because of the wide variation in body size of adult masu salmon (Machidori and Kato 1984). From the results of using marked salmon belonging to the same brood-year-class, a remarkable variation was seen in the adult body size. Only one marked fish returned to the river the next year as a 4-year-old fish that had spent two winters in freshwater and one winter in the ocean; thus it became clear that all masu salmon produced in the Shiribetsu River spent one year, or one winter, in the ocean returning afterwards to its natal waters for spawning.

An examination of the scale patterns of marked adults did not show any significant correlation between adult sizes and estimated smolt sizes at the beginning of the seaward migration (Ohkuma and Mayama 1985).

Adult trapping for artificial hatchery work in the Shiribetsu River was carried out from late August to mid-October with peak captures occurring in mid-September. A total of 361 marked masu spawners were recaptured in the river.

The estimated rate of return was 0.7% in the natal river, 5.8% in the coastal waters within 50 km from the river mouth: a total of 6.5% of the 60,000 smolt originally released (Table 1). The migration range of masu salmon in the ocean is extremely limited compared to that of other salmon (Machidori and Kato 1984). Masu salmon migrate along the coastal waters of Japan for 8 to 9 months outside the summer season when they stay in the Okhotsk Sea. During the summer period before the spawning return, fishing mortality is considered to be very high. It is also likely that a large number of young masu salmon encounter fishing nets during their northward migration.

Table 1
Estimated numbers of marked masu salmon returned in the natal river and coastal waters around the natal river, 1982. (Modified after Mayama et al. 1986b.)

Area	Estimated no. of fish returned	% of return to each area
River	404	0.7
Coast within 50 km from the mouth of natal river	3,564	5.8
Total	3,968	6.5

Body Size of Returned Adults from the Smolt Release

The size of spawning adults from the same release group varies extensively as mentioned above. Their fork length distribution is shown in Figure 4. The average male fork length was larger, and variance greater, than that of females in every river. There were obvious differences in fish size by area. The average fork length of fish caught in the Shari River on the Okhotsk Sea side was small, about 40 cm, which is 10–15 cm less than fish on the Japan Sea

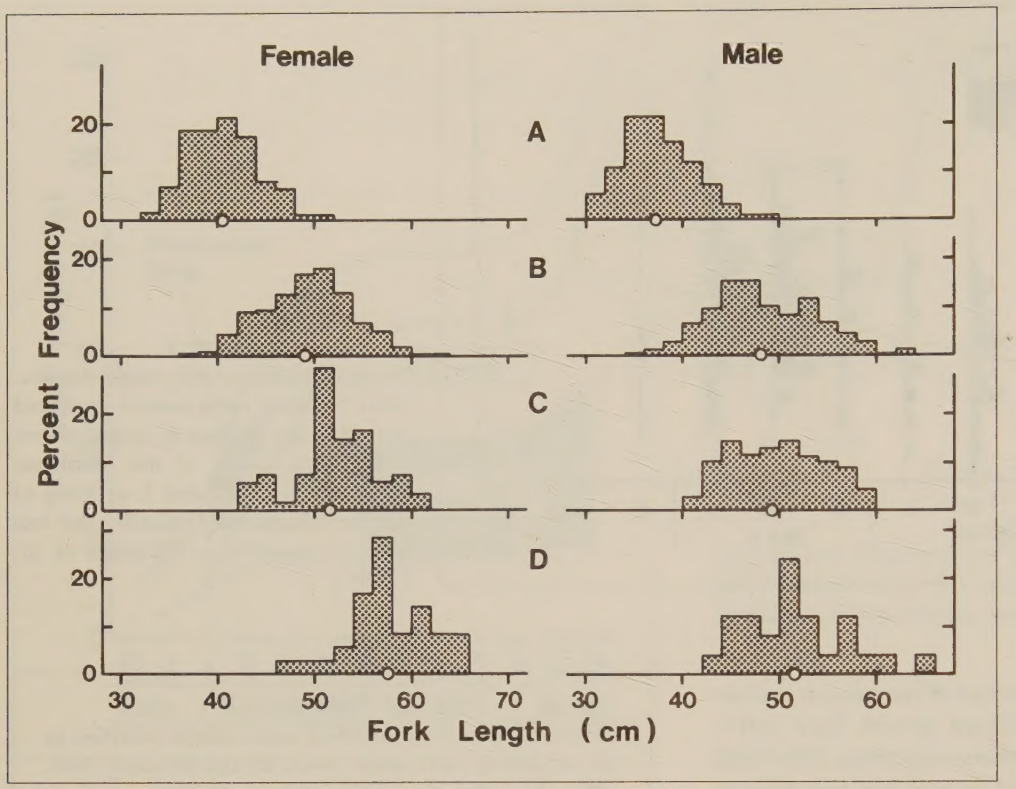

Figure 4
Fork length distribution of marked masu salmon recaptured in various rivers. (**A**) Shari River (Okhotsk Sea coast); (**B**) Shibetsu River (Nemuro Strait); (**C**) Shizunai River (Pacific Ocean); and (**D**) Shiribetsu River (Japan Sea).

side, which are on average the largest. Masu salmon in the Nemuro Strait and the Pacific Ocean sides were between these values.

According to past studies (Machidori and Kato 1984), the size of upstream migrating adults varies extensively by area in far-east Asia. The size of masu salmon caught in the Primore and northeastern Sakhalin areas are larger than those in the Okhotsk Sea side in Hokkaido. Thus, the relationship between latitude and adult size is not clear. Within the limits of these results, we do not have sufficient knowledge to explain the differences in fish size by area.

Growth of Hatchery-reared Fish after the Release

In order to clarify the influence of hatchery rearing upon growth and survival after release, the body size of returned adults and rate of return is useful information. However, as mentioned before, it is difficult to estimate the return rate, because the total catch of masu salmon during their feeding migration is not yet clear.

The relationship between average fork length of adults from fish released into rivers on the Japan Sea coast and of adults from wild smolts of the same bood-year-class is shown in Figure 5. These released fish were produced from the native stocks of the rivers used in these experiments. As shown in this figure, adults from the released smolts were always smaller than the wild fish. The differences were small at times, but usually there were significant differences. On the other hand, fingerlings, released in the fall before their overwintering, returned as equal to or larger than the wild fish (Mayama et al. 1988). These results indicate that smolts released after long-term rearing have difficulty acclimating to new environments. It is necessary to determine either the best time or the optimum smolt condition, to increase both the survival of masu salmon smolt and their adult size.

Effect of Transplantation on Adult Return

We confirmed that smolt releasing was an efficient method to establish an original stock rapidly. However, it is very difficult to take a large number of eggs on a regular basis from ascending native spawners. Salmon eggs have been transplanted frequently to various areas. In particular, almost all the chum salmon resource in Japan has been supported by artificial propagation so that a great number of eggs are transplanted among many rivers. But, in the genetic study of the chum salmon population structure by Okazaki (1982), the author suggested that it was nearly impossible to expect good results from a transplantation between latitudinally distant rivers. He also reported that high genetic divergence occurred among masu salmon river populations and that significant differences in the frequencies of alleles were observed even among the proximal river populations (Okazaki 1986).

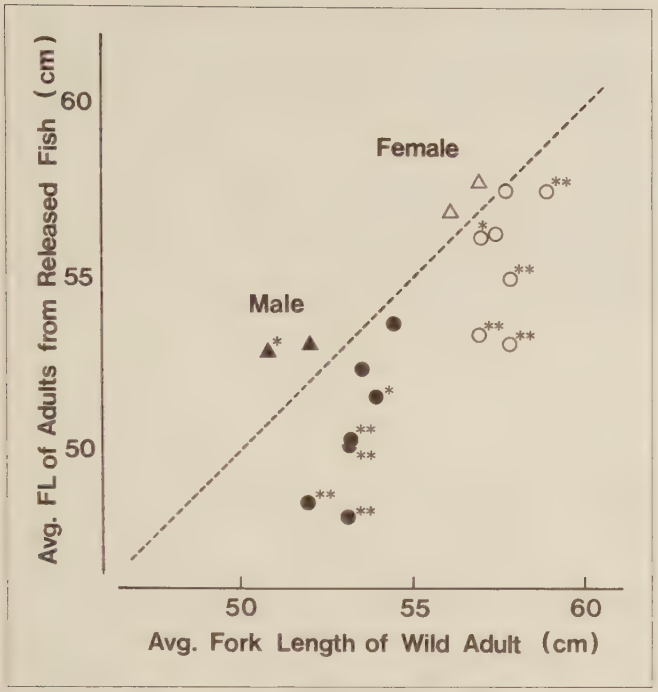

Figure 5

Relationship between the average fork length of adult masu salmon from hatchery-reared smolts (circles), or fall-releasing fish (triangles), and that of adults from wild smolts of the same brood-year-class. Shaded symbols indicate comparison of males, open symbols females. Asterisks indicate significant difference ($*P<0.05$; $**P<0.01$). (Data: Mayama et al. 1985b, 1986, 1987, 1988, 1989.)

As previously discussed (Fig. 4), there was obvious difference in fish size among various rivers. The Shari River stock in the Okhotsk Sea belongs to the minimum fish size group. In contrast, the Shiribetsu River stock in the Japan Sea belongs to the maximum size category. Those rivers are located a great distance from each other (Fig. 2). Exchange transplantation experiments between these rivers were carried out to clarify the ability of nonnative adults to return to the site of outplanting as adults.

Two groups of smolts, one from the native stocks and another from the introduced stocks were released at the same time into each river (Table 2; Mayama et al. 1989). Unfortunately, we cannot compare the differences in returning fish size between strains in each river because the number of adults recaptured in the rivers from the introduced stocks was very small. The recapture rate of the introduced group was significantly lower than the native group in both rivers. Differences in the seaward migration time were thought to be causing the low survival rate of the introduced stocks.

The smolts were collected repeatedly during their seaward migration (nine times at intervals of 1 to 4 weeks) at an area about 10 km downstream from the release site in the Shiribetsu River from April to July (Fig. 6). Captured fish were classified into smolt and parr by morphological features.

The seaward migration period of the hatchery-reared smolts originating from the native stock corresponded with that of the wild stocks. On the other hand, smolts from the introduced stock originating from the Shari River migrated downstream in June, or about one month later than the natives.

Table 2

Summary of experimental data for fish stocks exchanged between the Shiribetsu and Shari rivers and for native river stocks (modified after Mayama et al. 1989.

Brood year	Original river	Release: Date	Release: No. of smolts	Release: Avg. fork length (cm)	No. of adults recaptured: In coast	In river	Total	Recapture rate (%)[a]
Shiribetsu River								
1984	Native	22–23 Apr. 1986	25,000	12.4	86	58	144	0.58(0.23)
	Shari	23–24 Apr. 1986	37,000	9.9	13	4	17	0.05(0.01)
1985	Native	21–22 Apr. 1987	37,000	11.2	31	49	80	0.22(0.13)
	Shari	20 Apr. 1987	45,000	10.1	9	2	11	0.02(0.00)
Shari River								
1984	Native	13 May 1986	39,000	11.9	182	195	377	0.97(0.50)
	Shiribetsu	13 May 1986	35,000	14.4	29	8	37	0.11(0.02)
1985	Native	20 May 1987	42,000	12.4	36	345	392	0.93(0.82)
	Shiribetsu	20 May 1987	44,000	15.5	13	25	38	0.09(0.06)

[a]Recapture rate: smolts-to-total adults. Value in Parentheses: smolts-to-adults in river.

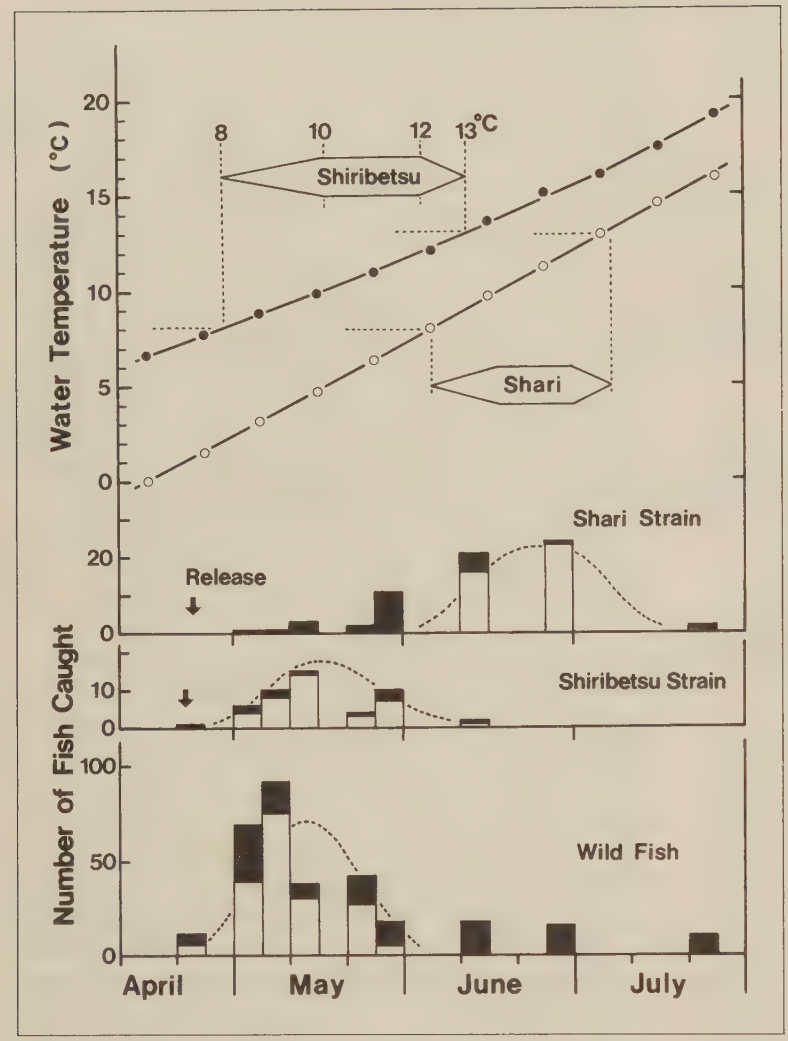

Figure 6

Seasonal changes in seaward migration of different stocks of masu salmon smolts released into the Shiribetsu River in 1986 and the surface water temperature in the adjacent coastal waters at the mouth of the Shiribetsu River (solid circle) and the Shari River (open circle), Optimum migration period (8–13°C) of each stock in its natal waters is shown as the range. Fish were caught in the lower reaches of the river and classified into smolt (open part of histogram) and parr (shaded part). (Modified from Mayama et al. 1989.)

In the coastal waters of the Japan Sea off south-western Hokkaido, it is known that the density of chum salmon fry decreases rapidly from late May to early June, disappearing in mid-June (Mayama 1985; Mayama et al. 1982, 1983). The seaward migration of masu salmon smolts is usually two or four weeks behind that of chum salmon fry. Masu salmon smolts reach the coastal waters when the surface water temperature is approximately 8 to 10°C. Both species of salmon disappear from the coastal waters in early June when the water temperature reaches from 12 to 13°C. The same migration pattern was observed off the Pacific coast of Hokkaido (Sano and Abe 1967).

It is not yet clear what triggers the mechanism that stimulates young salmon to disappear from the coastal area adjacent to the natal river. However, offshore migration may be induced by a simultaneous change in the marine environment which produces inadequate conditions for growth (Mayama 1985). The effect of the Tsushima Warm Current also increases at this time (Hata 1962). Some hatcheries raising chum salmon on the Japan Sea side have used low temperature (instead of warmer spring water) for rearing. The colder water retards development and leads to a longer rearing time and a later release date. The occasional absence of any returns under these conditions indicates high mortality as a result of delayed release (Mayama, unpubl. data).

Therefore, from the observations made on chum salmon, it is suggested that the low returns of introduced stock into the Shiribetsu River indicates high mortality as a result of a late seaward migration. The Shiribetsu River stock which was introduced into the Shari River smolted at the same time as the native stock; however, the introduced stock migrated to sea about one month earlier than the natives.

Along the Okhotsk Sea coast, drifting ice comes close to shore until mid-April. After the ice disappears, water temperatures increase reaching only 4 or 5°C by mid-May (Fig. 6) when the early smolt of the Shiribetsu River stock migrate to sea. The temperature may be too low for the smolt, but this is not considered as the critical factor in

their mortality. The smoltification peak of native Shari River smolts coincides with the occurrence of a temperature of 10°C in the Okhotsk Sea. Similarly, the same peak occurs with the native Shiribetsu stock as waters in the Japan Sea reach 10°C.

We do not have sufficient knowledge to explain why the early seaward migration of the Shiribetsu strain in the Okhotsk Sea side resulted in low survival. Many factors have yet to be considered, all of which influence the genetic adaptation of the stock to a new environment.

Conclusion

The effects of masu salmon transplantation from proximal rivers have been evaluated. The results indicate that preservation of the native stocks is the most important factor in their rehabilitation, and that a donor river must be selected using definite but yet undefined criterion for more effective transplantation. This theory is supported by results with coho salmon (*O. kisutch*) (Reisenbichler 1988).

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Recent Advances in Halibut (*Hippoglossus* spp.) Culture

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ABSTRACT

The Atlantic halibut (*Hippoglossus hippoglossus*) is currently receiving a significant amount of research attention in Norway, Iceland, and Scotland and interest in the culture of that species is developing in North America (particularly Nova Scotia and Newfoundland, Canada). Success in spawning and larval rearing has been achieved by Norwegian scientists though the numbers of postlarvae produced to date are small. On the west coast of the United States, our research in conjunction with the International Pacific Halibut Commission, the United States Fish and Wildlife Service, and the National Marine Fisheries Service has led to the captive spawning of Pacific halibut (*H. stenolepis*). We have also investigated seasonal patterns in circulating hormone levels. Future work with Pacific halibut will be aimed at production of postlarvae and determination of nutritional and environmental requirements of larvae and juveniles.

Introduction

Atlantic halibut (*Hippoglossus hippoglossus*) and Pacific halibut (*H. stenolepis*) have long been fished by various nations. Atlantic halibut are found in the boreal and subarctic Atlantic Ocean. Along the North American coast they have been occasionally reported as far south as New Jersey and New York, and in the eastern Atlantic they have been reported from the Bay of Biscay and the English Channel. The fish are abundant in the North Sea, near Iceland, along the Norwegian coast, and in the Barents Sea (Bigelow and Schroeder 1953). Pacific hailibut also have a broad distribution, occurring from California northward along the coast of North America into the Bering Sea. Along the eastern Pacific coast, *H. stenolepis* occur as far south as northeastern Japan (Hart 1973).

Management of Pacific halibut has a long history. The International Fisheries Commission (currently the International Pacific Halibut Commission or IPHC) was organized in 1924 and began its work early in 1925 (Thompson 1950). Today, the IPHC manages the commercial halibut fishery off the west coast of the United States and Canada, where yearly quotas in some areas are taken in 48 hours or less of fishing!

A great deal of information has been collected on the basic biology of halibut, but the interest of aquaculturists is largely a phenomenon of the present decade. While there are some differences between the Atlantic and Pacific species, they have many characteristics in common and it is assumed that much of the information generated by aquaculturists interested in one species can be applied to the other.

Biology of Halibut

Halibut are laterally compressed, assymetrical fishes with color occurring only on the right side. In postlarval and larger individuals the eyes both occur on the right side as is typical of members of the family Pleuronectidae.

Individuals may exceed 300 kg in weight (Bell and St. Pierre 1970). Halibut are strict carnivores which prey primarily on other fishes. Crabs, lobsters, squid, clams, and mussels are also frequently recovered from halibut stomachs (Bigelow and Schroeder 1953; Hart 1973).

The spawning season of Pacific halibut extends from November to late March, with peak spawning occurring in December and January (St. Pierre 1984). Spawning occurs near the continental shelf edge at depths ranging from 100 to 550 m. Pacific halibut males generally mature at eight years of age, while females typically mature at 12 years (St. Pierre 1984). Maturity in males generally occurs when the fish are between 70 and 110 cm, while females mature at lengths of 90–140 cm (Novikov 1964). Atlantic halibut spawn in the winter and spring at depths from 180–1000 m (Thompson and Van Cleve 1936). Water temperature for spawning of both species is within the range 3–8°C (Thompson and Van Cleve 1936).

Halibut have large eggs relative to other flatfishes. Forrester and Alderdice (1973) indicated that the mean unfertilized egg diameter for Pacific halibut is 3.1 mm. Atlantic halibut eggs average 2.9 mm (Lønning et al. 1982). Fertilized eggs expand over a period of a few hours to 3.2–3.3 mm for Pacific halibut (Forrester and Alderdice 1973) and 3.1 mm for Atlantic halibut (Blaxter et al. 1983). Egg development of both the Pacific and Atlantic species appears to be identical (Forrester and Alderdice 1973; Rollefsen 1935).

Larval development of Pacific halibut appears to be typical for the genus. Two to three weeks following fertilization, the drifting eggs hatch into pelagic larvae 8–15 mm in length (Thompson and Van Cleve 1936). Time to hatching is dependent upon temperature (Van Cleve and Seymour 1953). As larval development progresses, the fish move upward in the water column and over a period of three to five months are carried shoreward by surface currents. Following metamorphosis, postlarvae settle to the bottom in about May or June (Thompson and Van Cleve 1936).

Halibut Culture

Atlantic halibut have been spawned in captivity for many years in Europe (Meggs 1988). Spawning of Pacific halibut in the laboratory has also occurred in Canada (Forrester and Alderdice 1973). Adult Atlantic halibut are currently being held in Iceland, but spawning has not yet been accomplished (Arni Isaksson, Institute of Freshwater Fisheries, Reykjavik, Iceland, pers. commun., January 1990). After several attempts to produce postlarval Atlantic halibut, researchers at the Norwegian government laboratory at Austevoll, near Bergen, were successful in 1986. From two postlarvae produced that year, about 200 survived in 1987, and that number was expected to increase to 1,000 in 1988 (Meggs 1988). In 1987, the Sea Fish Industry Authority Marine Farming Unit in Scotland reportedly produced about 22,000 larval Atlantic halibut from 700,000 eggs and was able to carry 18 fish to a stage approaching metamorphosis (Anonymous 1988).

While the number of surviving postlarval halibut produced in captivity remains small, interest in aquaculture of *Hippoglossus* sp. is growing rapidly. Research or development of aquaculture enterprises centered around halibut is occurring in Norway, Iceland, Scotland, Canada, and the United States. We have spoken with investigators in Newfoundland, Nova Scotia, and British Columbia, Canada who are interested in halibut as an aquaculture species.

Discussions with Ingvar Huse (Institute of Marine Research, Austevoll Marine Aquaculture Station, Storebø, Norway, pers. commun.) both in Austevoll, Norway (September, 1987) and during a visit he made to Seattle, Washington (November, 1987) revealed that larval Atlantic halibut are maintained in total darkness. Exposure to light is related to increased mortality. The eggs and larvae are suspended in the water column and are easily damaged if they strike the walls of rearing containers. Buoyancy can be maintained if the proper combination of salinity and temperature is provided.

In 1980, spawning adults were captured by gill net at sea and stripped aboard ship (Blaxter et al. 1983). In recent years, captive brood stock have been maintained at Austevoll and are the primary source of gametes. The eggs, which are incubated in the dark, are neutrally buoyant at 37‰ salinity at 5 and 7°C. Hatching occurred after 18 days at 5°C and 13 days at 7°C. The presence of antibiotics in the culture water led to improved percentage of hatch.

In their first attempts at halibut rearing, Austevoll researchers had little success in getting fry to feed after yolk-sac absorption. However, that situation has improved. First-feeding larvae are offered wild zooplankton in Norway (Ingvar Huse, pers. commun.) and cultured copepods (*Trigriopus* sp.) in Scotland (Anonymous 1988). Postlarval Atlantic halibut can be trained to accept pelleted feed (Ingvar Huse, pers. commun.).

Our work in Washington is centered around determination of some of the basic biology of larval and juvenile halibut, but the results of that research clearly have aquaculture implications. Our research is supported by the International Pacific Halibut Commission and is being conducted cooperatively with the National Marine Fisheries Service and the U.S. Fish and Wildlife Service. The latter has facilities at their Marrowstone Island facility on northern Puget Sound (Fig. 1).

Limited success has been achieved in spawning and larval rearing of Pacific halibut. The techniques employed are similar to those developed in Norway for Atlantic halibut. Brood stock are collected at sea and transported in live tanks to the Marrowstone laboratory where they are

Figure 1
U.S. Fish and Wildlife Service laboratory on Marrowstone Island, Washington, a location for halibut research by the authors.

placed in a circular rubber lined tank about 5 m in diameter and 1.2 m deep. Pacific halibut rapidly adapt to the holding tank. They begin feeding actively within a few days. The broodfish tank can easily accommodate 20 broodfish of 60–100 cm length.

A black plastic cover is kept over the brood stock tank to drastically reduce incident light levels. The fish are provided with fresh or frozen Pacific herring (*Clupea harengus*) two or three times a week.

During the past two years we have had only one mature male available. The duration of the spawning season was shortened because that fish became ripe before the females and ceased the production of viable sperm before some of the females had become ripe. Short-term storage of sperm in the refrigerator was accomplished, and a recently published report indicates that it is possible to use cryopreservation on the sperm of Atlantic halibut (Bolla et al. 1987). If problems with males occur in the future, we will attempt to freeze sperm for later use, using the cryopreservation technique.

For the 1988–89 spawning season, additional males were to be obtained. There has been no problem getting adults to mature in captivity, though Liu (1988) collected data indicating that females may not spawn each year after reaching maturity.

During the 1986–87 spawning season, spontaneous egg release in the brood stock tank occurred, but eggs were also obtained by stripping. No fertilization was achieved. However, in the 1987–88 spawning season, several groups of eggs were obtained from five females, and sperm was obtained from the single available male. Approximately 10,000 eggs were fertilized, from which a total of eight larvae were produced. This appears to have been the first time that intentional captive spawning of Pacific halibut was achieved in the United States. Spontaneous spawning, subsequent stripping and fertilization, followed by larval hatching was accomplished in British Columbia, Canada (Forrester and Alderdice 1973), but no long-term larval rearing was attempted.

None of the larvae hatched in 1988 were retained past five days because the numbers were so small. Hatching was achieved in chambers which allowed the eggs to rest on the surface of 150-micron mesh plankton netting that had been attached to one end of a short piece of plastic pipe

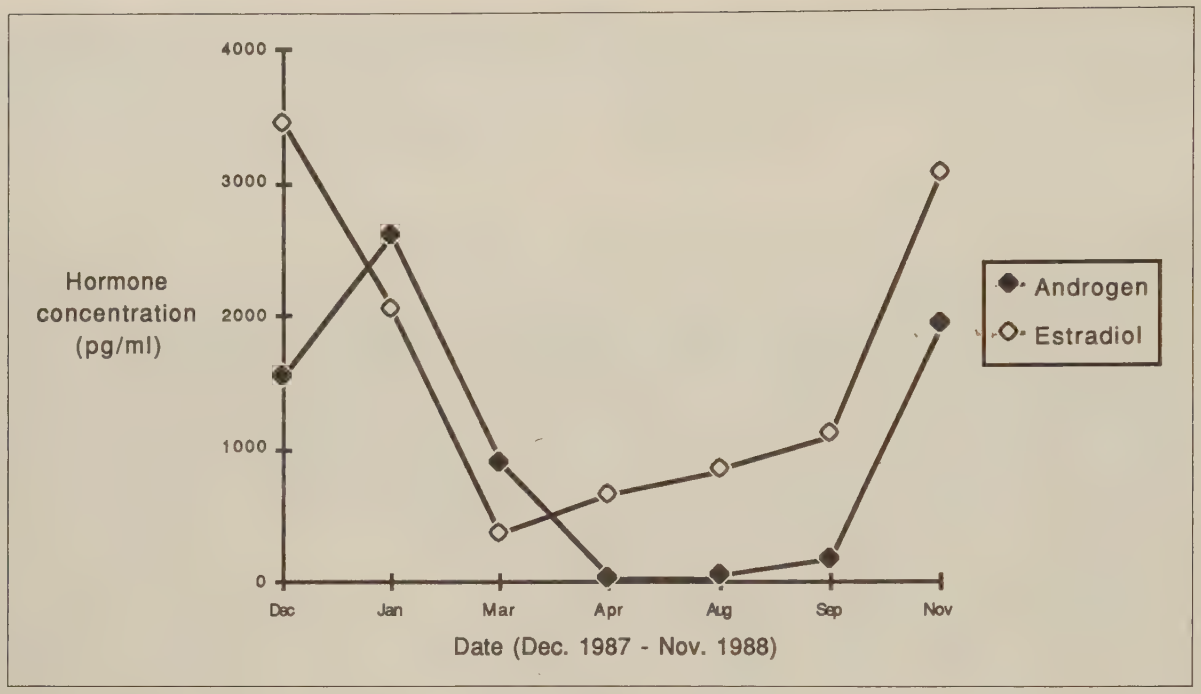

Figure 2
Mean seasonal patterns in plasma androgen and estradiol levels in mature female Pacific halibut.

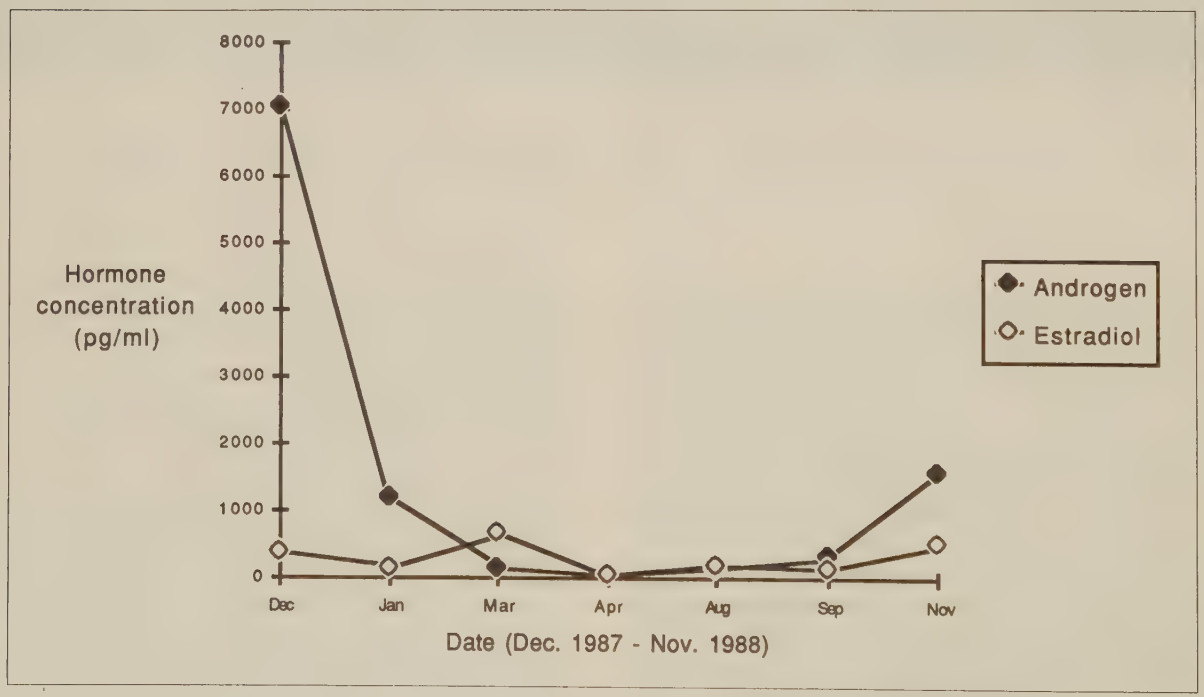

Figure 3
Seasonal patterns in plasma androgen and estradiol levels in a mature male Pacific halibut.

about 10 cm in diameter and placed within water baths that received a slow exchange of sea water. A variety of incubator designs were to be tested during the coming spawning season.

Initially our plans were to feed Pacific halibut fry on the rotifer *Brachionus plicatilis*. Brine shrimp nauplii (*Artemia* sp.) were to be substituted once the halibut larvae were too large to feed on rotifers. Prepared diets were to be used subse-

quently, the formulation being similar to salmon rations but with a reduced lipid content.

Blood samples were collected from captive Pacific halibut (4–6 females, 1 male) at intervals of several weeks from December 1986 through November 1987. From those samples, annual patterns in plasma androgen and estradiol levels were examined using radioimmunoassay (Sower and Schreck 1982). In mature females, steroid concentrations began to rise in September and peaked in December and January (Fig. 2). Steroid levels dropped dramatically a month before the fish spawned (each point in the figure represents the mean of samples obtained from four to six fish).

In the mature male, the androgen level began to increase in August and peaked in December, after which the concentration of that steroid declined precipitously about a month before mature sperm were obtained (Fig. 3). Estradiol was found in the male blood plasma, but little change in the level present occurred during the year, a pattern that was seen for both steroids in samples obtained from immature fish (Liu 1988).

In conclusion, our results have demonstrated that Pacific halibut broodstock can be held in captivity for extended periods, and that captive spawning can be achieved during the normal spawning season of the species. Viable larvae are difficult to obtain in large numbers, but modifications in the hatchery aimed at providing a more conducive environment for the fragile young fish are being made. The challenge of carrying larval Pacific halibut through to metamorphosis is currently being addressed.

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Control of Hydraulic Environment and Development of an Artificial Pocket Beach in Sandy Coastal Areas

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ABSTRACT

The control of shellfish larva dispersion is important for the establishment of shellfish enhancing grounds. The larva should stay within a planned area of the sea. The retention effect of pocket beach formation was studied with wave basin experiments and numerical simulation. A circulation in the pocket, created by wave shoaling, is important for water quality conservation. To intensify the circulation a wide entrance is needed, although for the retention effect the entrance should be narrow.

Introduction

Japanese fisheries are keenly interested in the development of the sandy coastal areas that comprise one third of the Japanese coastline of about 10 thousand kilometers.

A sandy coast confronting an open sea is an area where waves, coastal currents, and sand drifts are the prevailing forces which result in the creation of severe conditions for the survival of juvenile fish and shellfish. The natural area is therefore not left useful for fisheries purposes. Nevertheless, it happens that we found the enhancement of bivalves in harbors constructed in such a sandy coast (e.g., Japanese surf clam, *Pseudocardium sybillae*, in Hachinohe Port, Aomori Prefecture and *Meretrix lamarkii* in Kashima Port, Ibaraki Prefecture) while these ports were under construction. This suggests that we can enhance the marine resources on sandy coasts by controlling the flow of water and the drifting of sand.

In the Marine Ranching Program, we have accomplished the following types of environmental control for mariculture:

- development of a wave-induced circulation method (Toda and Nakamura 1981),
- diffusion control behind a wall (Toda 1983),
- fundamental study on internal hydraulic bore (Noma 1986),
- bed materials control in a sandy beach (Uekita and Akeda 1985),
- analysis of bottom flow pattern on a reef (Akeda and Uekita 1987),
- environment control of underwater forest,
- development of a "pocket beach" method (present paper), and
- design criteria for underwater structures (Takeuchi 1987).

This paper introduces the artificial "pocket beach", especially its retention effect on floating matter and current dispersed substances.

Control of the Hydraulic Environment in Sandy Coastal Areas

On sandy coasts, we sometimes find topography such that a small inlet is formed with sides composed of headlands of rock or reef (called a "pocket beach") whose sandy bottom is stable under severe conditions. One of the purposes of the study was to develop a method of creating an artificial pocket beach, where the controlled diffusion of substances and removal of bed materials occurs. Marine organisms spend their early larval stage floating and are transferred soley by the motion of seawater. This may result in a wider distribution of the species. On the other hand, it may result in a critically high mortality rate at that stage. Dispersion control aims to limit the motion of seawater, and thus, the larvae of target species, within a planned area.

Since the shallow water of sandy coasts is a severe dispersion field caused by wave and water flow, it is possible for the larvae to be transported to other waters or offshore.

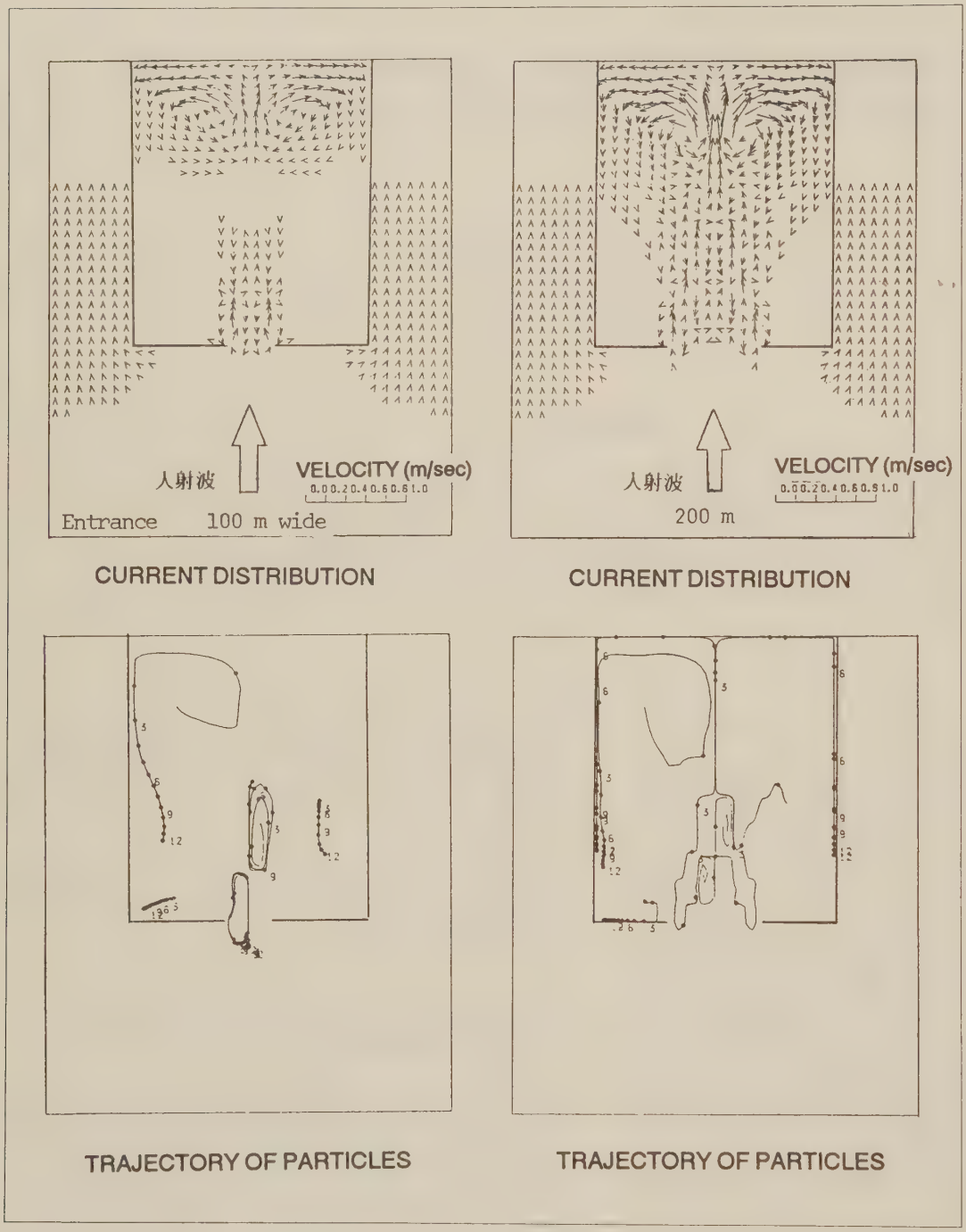


Figure 1
Simulated current distribution and trajectory of imaginary particle. Wave height is 1 m; wave period is 6 seconds.

So as not to be transported, the larvae must in some way be enclosed. A simple enclosure with four walls, for example, causes the seawater movement inside to cease and become stagnant, thereby causing mortality of the larvae. A protected enclosure is needed, which results in the formation of a circulation. An eddy or circulation can be independent of a general flow such as coastal current and can prevent dispersion.

Offshore breakwaters are one of the coastal structures used to attenuate wave energy and to control the movement of bed materials. In mariculture, offshore breakwaters are adopted for the purposes of

- attenuating wave action,
- allowing for continued seawater exchange,
- providing dispersion control (i.e., suppression of longshore current and rip currents), and
- controling drifting sand.

Figure 1 shows a simulated flow pattern in the pocket beach on the bottom with slope 1:100, the water depth at

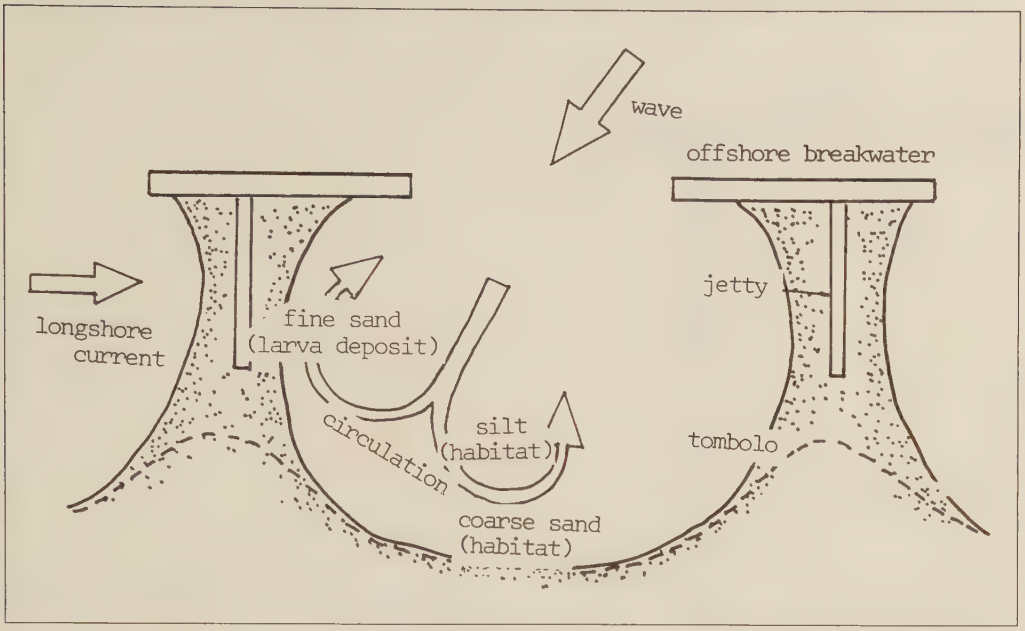


Figure 2
Schematic presentation of pocket beach.

the entrance is 6 m. The simulation principally consisted of a calculation of the wave field, a calculation of flow field, and consideration of the movement of the sand bed. In the simulation, the last factor was omitted.

A circulation can be intensified with submerged dikes, because the breaking of a wave beyond the dike induces a large flow velocity in the direction of the propagated wave. If the circulation control method is performed in an area enclosed by three sides, the suppression of the dispersion is greater than that of simple arrangement of an offshore breakwater. This area may be used as a nursery ground in sandy coastal areas. The mechanisms by which bivalve establishment are enhanced in a pocket shaped beach as shown in Figure 2 is interpreted as follows:

- suppression of the dispersion of larvae,
- supply of proper habitats for newly settled shellfishes because of segregated bed materials by wave action into coarse, fine, and silty sands.
- calmness (i.e., reduced turbulence),
- wave-induced circulation to prevent stagnation of water, and
- accumulation of larvae from outer waters owing to mass transportation by waves and currents.

Hagino (1987) investigated the ability of structures to suppress the dispersion of retention of free swimming organisms by wave basin experimentation and numerical simulation. Figure 1 is a simulation example.

Outline of the Experimentation

In an experimental wave basin as shown in Figure 3, the model pocket was settled on a bottom with slope 1:100, length and width of approximately 6 × 3 m, with entrances of either 1- or 2-m diameter. Incident waves of 1 and 3 cm high with periods of 0.6 and 1.0 sec, respectively, were made. Wave height was measured by a conductance wave gauge, flow velocity by an ultra-sonic flow meter, and dye concentration change (dye was injected and fully mixed inside the pocket before wave test) by a photo-electric colorimeter.

Six wave gauges were equipped on an observation truck, and measurements were done in planned points using a 100 × 100 cm mesh. Three flow meters and three colorimeters were set on the bottom of the basin; planned meshpoints were also measured. That is, Figure 4 represents the compound results of repeated tests under the same experimental conditions. Station number (St.) in the figure were points where dye concentration was measured.

The dispersion characteristics were mainly analyzed by dye concentration change. In the numerical simulation, the motion of an imaginary particle was determined and compared with the basin experiment.

Results

Figure 4 shows the results of the basin tests with entrances of width 1 and 2 m, the arrows representing the flow velocity vector, and broken lines representing wave height distribution. "Case" represents experimental conditions, and the difference between Cases 6 and 8 was entrance width of the pocket. In both cases

$$\text{incident wave height } H_0 = 3.8 \text{ cm},$$

and

$$\text{wave period } T = 1.3 \text{ sec}.$$

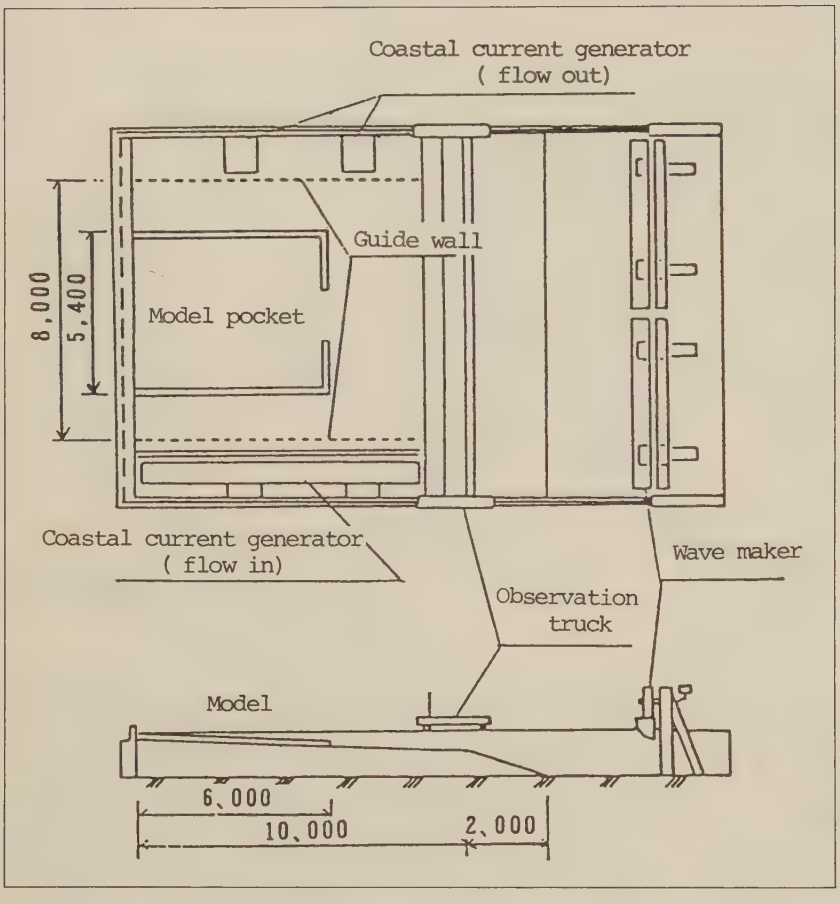
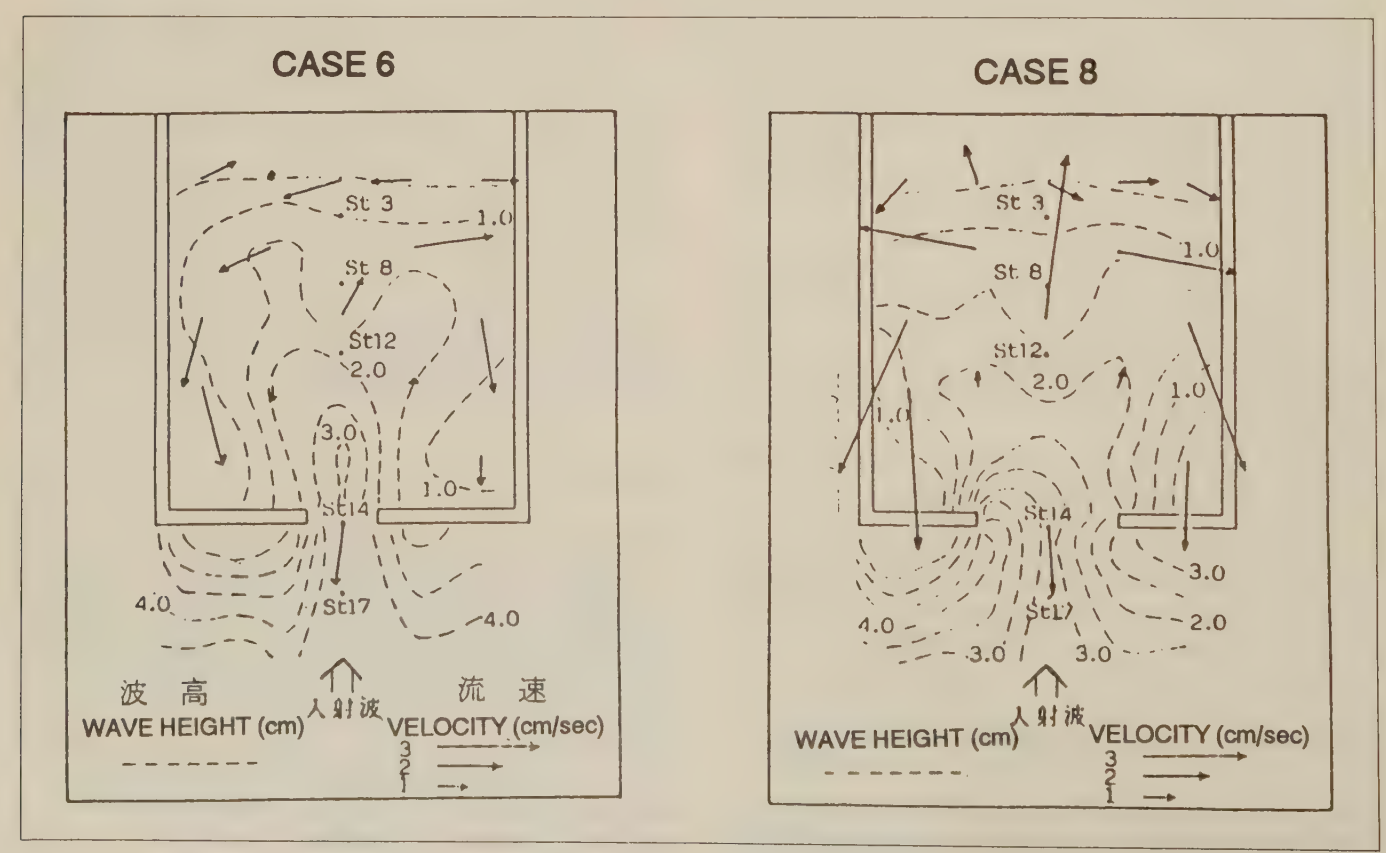


Figure 3 (left)
Experimental basin and apparatus. Units are in millimeters.

Figure 4 (below)
Current vectors and wave height. Case 6 entrance is 1 m wide; Case 8 entrance is 2 m wide.



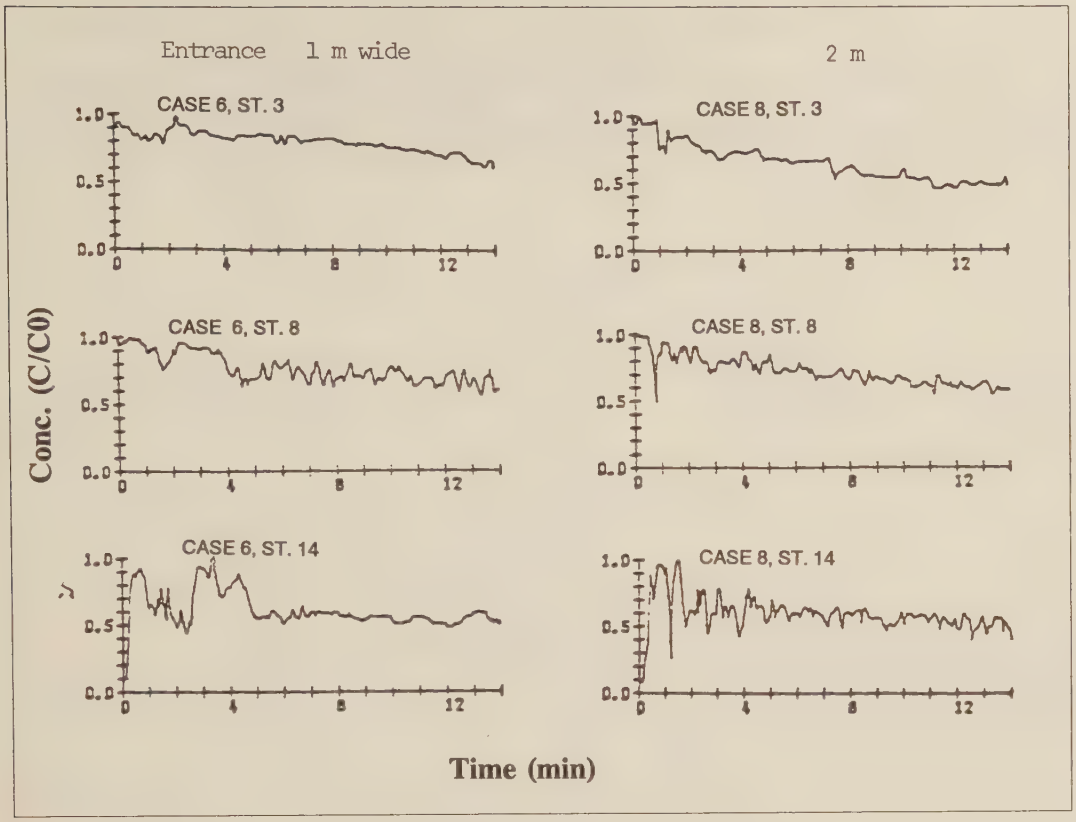


Figure 5 (left)
Time change of dye concentration.

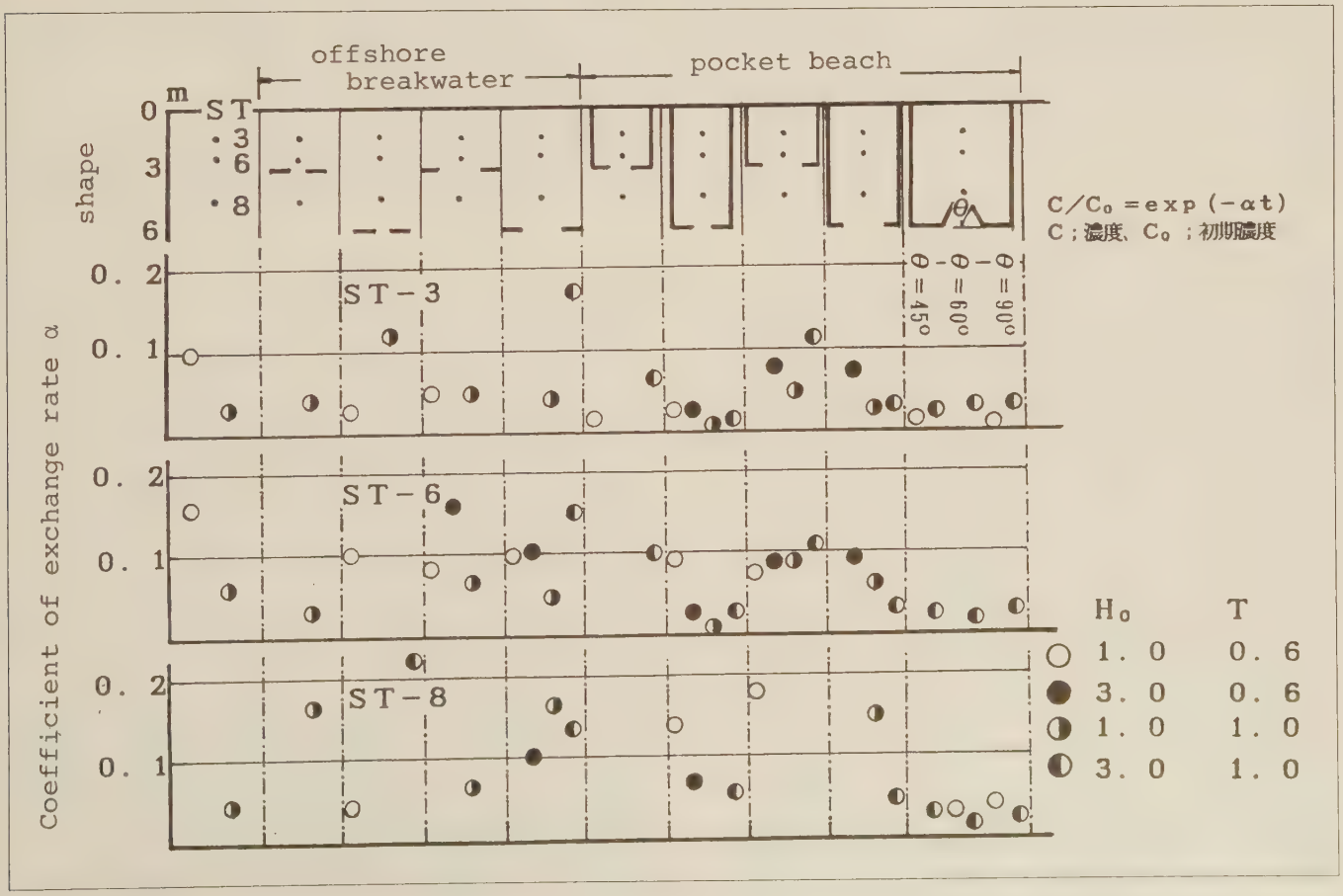


Figure 6 (below)
Measured exchange rate at each measuring point. H_0: incident wave height (cm); T: wave period (sec).

Flow velocity with the 2-m wide entrance was about twice as fast as that with a 1-m wide opening.

Figure 5 shows the changes in dye concentration. The concentration decreases gradually at each measuring point by mixing with noncolored water outside the pocket. Assuming that perfect mixing occurs inside the pocket, the dye concentration can be estimated by Equation 1:

$$C = C_0 e^{-\alpha t} \quad (1)$$

where, c is the dye concentration at time t, c_0 is the initial concentration, and α is the coefficient of seawater exchange rate. Coefficient α is determined by the rate at which dye concentration decreases according to the formula:

$$\alpha = \frac{1}{R_t}.$$

The value R_t is obtained experimentally as shown in Figure 6. If the retention time of the dye, R_t is defined as the time elapsed until the initial concentration, c_0 decreases to c_0/e.

Conclusions

In a pocket beach, waves produce circulation, the intensity of which relates to entrance width, wave height, and wave period. The wider the entrance, the stronger the circulation. For the retention time of a unit volume of water, narrowing the entrance improved dispersion control.

Deployment of this method was planned for enhancement of *Meretrix lamarkii* in Aomori Prefecture but it has not yet been done owing to problems with the cost/benefit ratio.

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Temporal and Geographic Variability in Survival of Sea-Ranched Coho and Chinook Salmon in North America

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ABSTRACT

Public, private, and cooperative ranchers released about 1.9 billion salmonid fingerlings from North American hatcheries into the eastern Pacific Ocean in 1987. This number has increased from a level of approximately 700 million since 1982, largely owing to major increases in pink (*Oncorhynchus gorbuscha*) and chum (*O. keta*) production in Alaska. Chinook (*O. tshawytscha*) and coho (*O. kisutch*) salmon continue to represent the majority of production on a weight basis because of their relatively large size at release, often exceeding 40 g. Chinook and coho salmon have historically been emphasized in public sea ranching because of their importance to North American recreational and commercial fishermen. As a result, relatively more is known about culture techniques, ocean survival, and the contribution to fisheries of these species. Over 25 million of these salmon are marked each year before release to increase our knowledge of survival and contribution rates. Results from marking programs have shown that survival rates after release to the ocean are highly variable, ranging from near zero to over 30% for coho salmon. Research has shown that survival and contribution rates vary for a number of reasons including geographic and temporal influences. Research has also detected significant variability in survival and contribution rates within production from a single hatchery and even within a single production lot. The paper will describe the variability found in contribution and survival rates of ocean-ranched chinook and coho salmon. Proposed sources of variability will include geographic and temporal causes as well as variability resulting from other sources.

Introduction

Along the Pacific Coast of North America, the use of hatcheries to produce juvenile salmon that feed and mature in the North Pacific Ocean is a traditional method of salmon enhancement (Allee 1988). Salmon ranching in the Pacific Ocean began at the McCloud River Hatchery, California in 1872 (McNeil 1980). By the late nineteenth and early twentieth centuries salmon hatcheries were operating throughout the eastern Pacific range of salmon. Early hatcheries focused most often on chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*O. kisutch*), and sockeye salmon (*O. nerka*), all of which normally require extended rearing in fresh water before migrating to sea (McNeil 1980). By 1987, releases of chinook and coho salmon on the Pacific Coast of North America had reached 154.4 million and 373.2 million, respectively (Allee 1988).

Coho and chinook salmon are exploited in the common property fishery by commercial and recreational fishermen and are most often taken in high seas and mixed-stock fisheries remote from their place of origin. Management of these fisheries is extremely complex. The fisheries must be regulated to assure adequate spawning escapement and to meet various harvest expectations. A good deal must be known about the performance of these species after they are released from the hatchery to migrate to the ocean. The salmon culturist must also have a means to evaluate various fish culture techniques and determine their effects on performance once the salmon are released from the hatchery.

An estimate of total survival is a parameter often used to describe the performance of ocean-ranched chinook and coho salmon. The estimate is usually stated as the percent of juveniles released to the ocean that are subsequently recovered in the fisheries and spawning escapement combined.

Survival rates are determined from salmon marking programs that have been conducted throughout the history of ocean ranching. Marks of various types have been

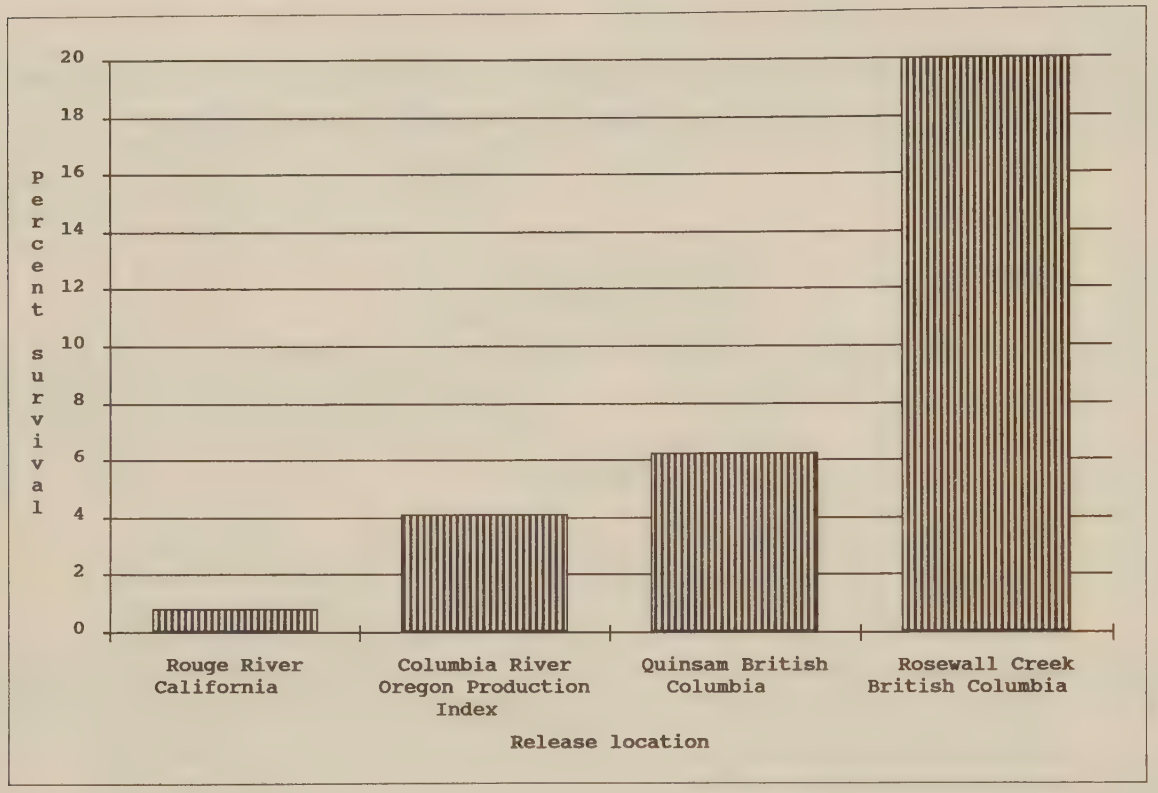

Figure 1

Survival of coho released at four locations between 38° and 51° N latitude. Sources: Pacific Fishery Management Council 1987 for Rouge River, California, and Columbia River, Oregon Production Index; Bilton et al. 1984 for Quinsam, British Columbia; and Bilton 1980 for Rosewall Creek, British Columbia.

recovered in the coast-wide fisheries and at the hatcheries as adult salmon return to spawn. Because chinook and coho salmon have historically been emphasized in ocean ranching, a large amount of survival data has been collected and relatively more is known about the performance of these species compared to other ranched salmonids. About 25 million salmon are marked each year. The primary tool used for marking salmon for evaluation is the implanted coded-wire tag (Bergman et al. 1968).

Upon examination of survival data, one fact becomes obvious. There is a wide range of variability in the post-release survival rates of ocean-ranched chinook and coho salmon. Survival rates vary with some consistency over geographic distances. Survival rates also vary temporally within geographic zones. There is also variability in survival rates within single production units and even within single experimental lots.

Geographic Variability

The range of chinook and coho salmon in North America extends from central California in the south to Kotzebue Sound, Alaska in the north, a distance of well over 3500 km (Wahle and Pearson 1987). Ocean ranching is practiced throughout this range with varying success. There is sufficient biological data on chinook and coho salmon to suggest that average smolt-to-adult survival can vary widely and in a consistent manner over geographic distances. For example, Bilton (1982) and Bilton, et al. (1984) report average smolt-to-adult survival rates of 25% for coho in the Georgia Strait region of British Columbia, Canada. Bilton and Jenkenson (1980) reported a survival rate of 47.5% for a group of coho released from Rosewall Creek Hatchery on Vancouver Island, B.C. Bilton (1980) reported survival rates for 72 groups of 1975-brood coho released from Rosewall Creek. The average survival rate for all groups combined was 19.99% (Fig. 1). In another study of coho smolts released from Quinsam Hatchery, B.C. in 1980, the average survival rate for 36 groups was 6.24% (Bilton, et al. 1984) and a maximum survival rate of 11.2% was predicted under optimum release conditions.

In contrast, survival rates for California and southern Oregon hatchery coho salmon were estimated over a 17 year period (PFMC 1987). The average rate for the period was 0.78%, ranging from 0.03% to 1.83% (Fig. 1). The average survival rate for Columbia River-origin coho salmon was estimated at 4.07% for the same period of time.

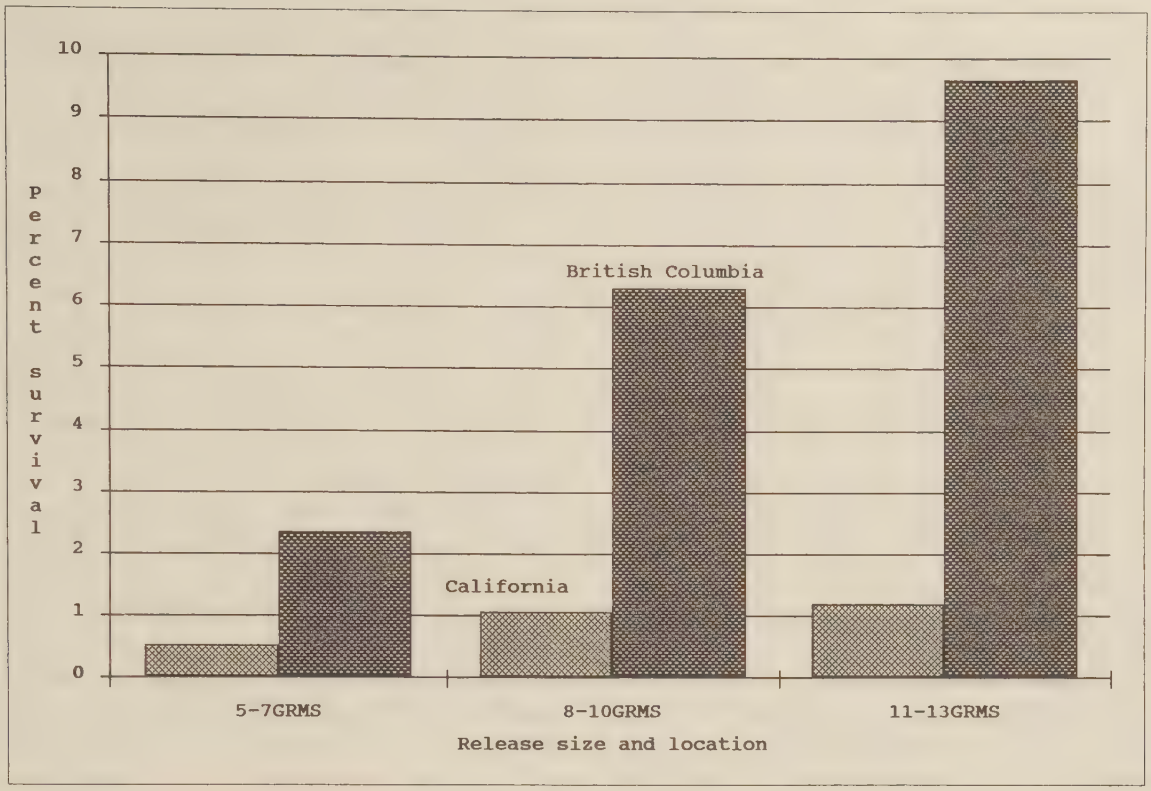

Figure 2

Survival of chinook salmon of three size categories released at 38° and 51° N latitude. Lighter shading represents three California hatcheries on the Sacramento River; darker shading represents the Big Qualicum Hatchery, B.C. Data for the 11-13 gram range represent only one point. Sources: Reisenbichler et al. 1982 and Pacific Fishery Management Council 1987 for California data; Bilton 1984 for Quinsam, B.C. data.

The specific causes for clinal differences in survival rates are not clear. One major factor, however, deserves speculation. The British Columbia, Canada hatcheries are located near the center of the range for coho salmon at approximately 51° N latitude. Coho salmon from California and southern Oregon hatcheries are produced near the southern extreme of the range, at about 38° N latitude. Coho salmon from the Columbia River are between the two at 46° N latitude. One might speculate that performance and productivity decline as a species nears the limits of its range. This factor might explain, in part, the progressive increase in survival rates of coho salmon between the southern limit and center of their range. If in fact there is a geographic cline affecting survival rates, as the data suggest, the need remains to explain the causitive mechanisms. For example, environmental and climatic factors are often implicated in the advance or decline of a species' range (Krebs 1972).

Alternatively, the differences in survival rates occurring over the range of coho salmon might simply be an artifact of poor hatchery practices, improper stock selection, localized climatic events, unique geographical features, or other direct causes. Those effects are less persistent than would be expected in the range-limited theory above.

Although less dramatic, there is evidence that the post-release survival of chinook salmon varies along a similar geographic cline as coho salmon (Fig. 2). The data are not as abundant but results of two studies might serve to suggest this trend.

Reisenbichler et al. (1982) compiled survival data for chinook salmon released from three Sacramento River (California) hatcheries at various sizes over several years. These data when compared to survival data for chinook salmon released at equivalent sizes and dates from Big Qualicum Hatchery (B.C.) (Bilton 1984) indicate a four to five fold difference in average survival rate. Again, chinook salmon produced from a British Columbia hatchery tended to have higher average survival than chinook salmon produced nearly 1500 km to the south.

It seems reasonable to speculate that these results are consistent with a theory of declining performance of a species near the end of its range. We must also note in this case, that the environment that chinook salmon experience after release from British Columbia hatcheries is relatively undamaged compared to the Sacramento River system in California. Hydroelectric projects, irrigation and domestic diversions, low flows, high temperatures, and toxic residues

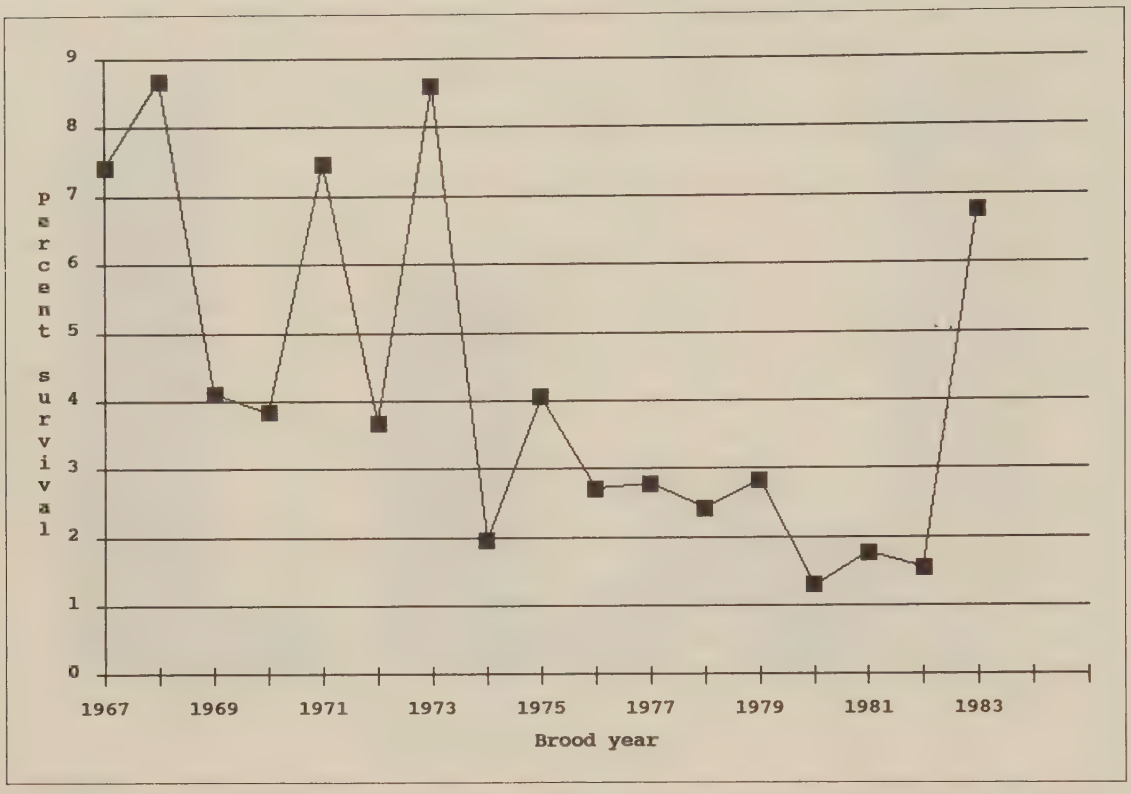

Figure 3
Estimated survival rates for public hatchery coho from the Oregon Production Index. Source: Pacific Fishery Management Council 1987.

all are present to a much greater extent in the Sacramento system. The Columbia River system is also affected—to a lesser degree than the Sacramento—but to a greater extent than in British Columbia. If there is truth to the speculation that survival decreases near the extreme of the range, then a low quality in-river environment might simply worsen an already tenuous existence.

Temporal Variability

Temporal fluctuations within many animal populations are well documented phenomena (Krebs 1972) and can be demonstrated for several populations of chinook and coho salmon. For example, the Chinook Technical Committee of the Pacific Salmon Council recently developed trend information for ten populations of ocean-ranched chinook salmon from British Columbia, Washington, and Oregon using a continuous series of coded-wire tag recovery data (Mike Fraidenburg, Wash. Dep. Fish., Olympia WA, pers. commun., Sept. 1988; *see* Appendix Figures). These trend lines represent the surviving cohort of 2- and 3-yr-old chinook salmon for a series of brood years. In general, the highest survival rates for chinook salmon are below the maximum rates for coho salmon. Even so, the survival rates within these populations of chinook salmon vary by a factor of eight to ten times over a ten year period, demonstrating that significant temporal variation is a common trait of salmon populations.

Continuous mark or tag data of this nature have historically been difficult to develop from marking or tagging studies because, by design, marking studies are often short term and discontinuous. However, adequate representation of long-term trends can be developed using historical records of landings coupled with escapement data, and when appropriate, stock composition data. One such data set (Fig. 3) provides a recent and notorious example of temporal variability in a coho salmon production unit. The Oregon Production Index (OPI) is a coho salmon production and harvest area where hatchery coho salmon of Columbia River origin predominate in the harvest from Monterey Bay, California to Leadbetter Point, Washington (Gunsolus 1978). A controversy erupted when the 1977 ocean commercial harvest in the OPI fell to the lowest point in 15 years. Ocean sport harvest dropped to a historical low. This collapse followed a record high production year in 1976. The controversy was compounded by the fact that adult production in the OPI was becoming increasingly erratic as hatchery production releases increased from fewer than 1 million coho smolts in 1960 to over 30 million

by 1970. By 1981, smolt releases totalled 61.9 million. During the period after 1970 the abundance of coho salmon appeared to fluctuate independently of the number of smolts released (Nickelson 1986).

The fisheries that exploit coho salmon in the OPI were severely impacted during years of low abundance, resulting in a flurry of activity among scientists attempting to explain the wildly fluctuating survival rates. Two primary hypotheses have been advanced in explanation. Several researchers, among them McGie (1981) and McCarl and Rettig (1983), hypothesized that abundance of coho salmon in the OPI was density dependent and that marine survival has been reduced by the release of excessive numbers of hatchery smolts. Peterman and Routledge (1983) and McGie (1984) suggested density dependent relationships in years of low coastal upwelling. In contrast, Clark and McCarl (1983) did not find evidence of density dependence. Nickelson (1986) concluded that survival in the OPI was density independent and proposed the second primary theory; that survival was correlated mainly with the strength of ocean upwelling in the spring and summer of smolt outmigration. He also presented evidence that ocean temperatures had an effect on survival but only in years of high upwelling.

At this time there is no final agreement on what has caused survival rates for coho salmon to vary so drastically in the OPI. We can be assured that new theories will be advanced and existing theories will be re-evaluated. It is probable that the complete explanation is complex and numerous factors are involved. It is also probable that the most influential factors, such as ocean upwelling, are beyond our control and any improvements we can make in hatchery practices will do little to dampen the magnitude of temporal variability over the long term.

Conclusion

Survival data for Alaskan chinook and coho salmon have not been included because it is difficult at this time to obtain compiled general survival data. These data, when available, will be important to the interpretation of clinal effects in geographic variability by addressing the northern extreme of the range for chinook and coho salmon.

It has proven difficult in general to find and use complete and comparable data for geographic or temporal comparisons. Marking and tagging projects often address a unique experimental situation over a short time period.

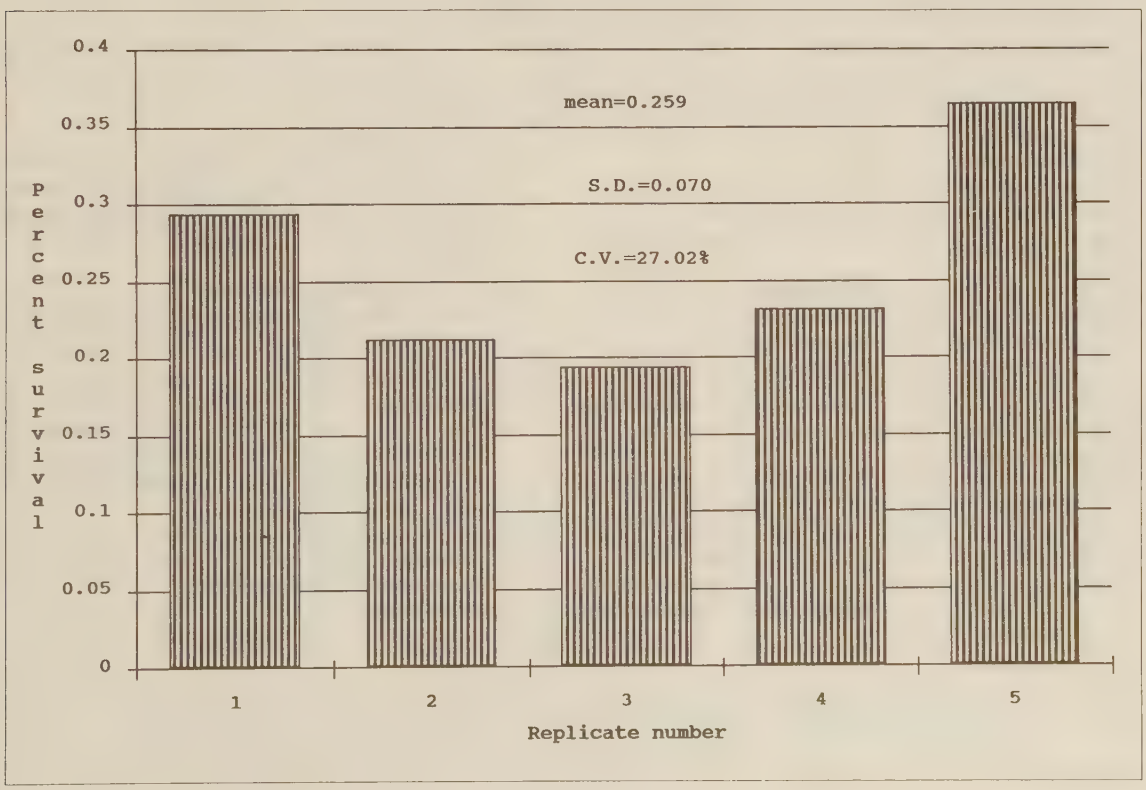

Figure 4

Observed variability in survival rates among replicates of a single experimental treatment group of Washington coho salmon at the Washougal Hatchery (A. Appleby 1984).

The results are not generally applicable on a broad geographic basis and are discontinuous so they do not support long-term temporal analyses. However, the results from unique short-term experiments do demonstrate that the level of variability even within statistical replicates of a single experimental treatment can be significant (Fig. 4). If researchers are to achieve better resolution of the extent and causes of variation in survival of ocean-ranched salmon a more extensive survival record must be developed. An effective tagging program would include annual application of tags to ocean-ranched salmon throughout the geographic range of interest and under similar rearing and release conditions. Only when variability in survival is shown to be persistent in magnitude and direction on a clinal basis or consistently related to causitive factors on a temporal basis and greater than the background variability within a single production group can the speculations made here be demonstrated in fact.

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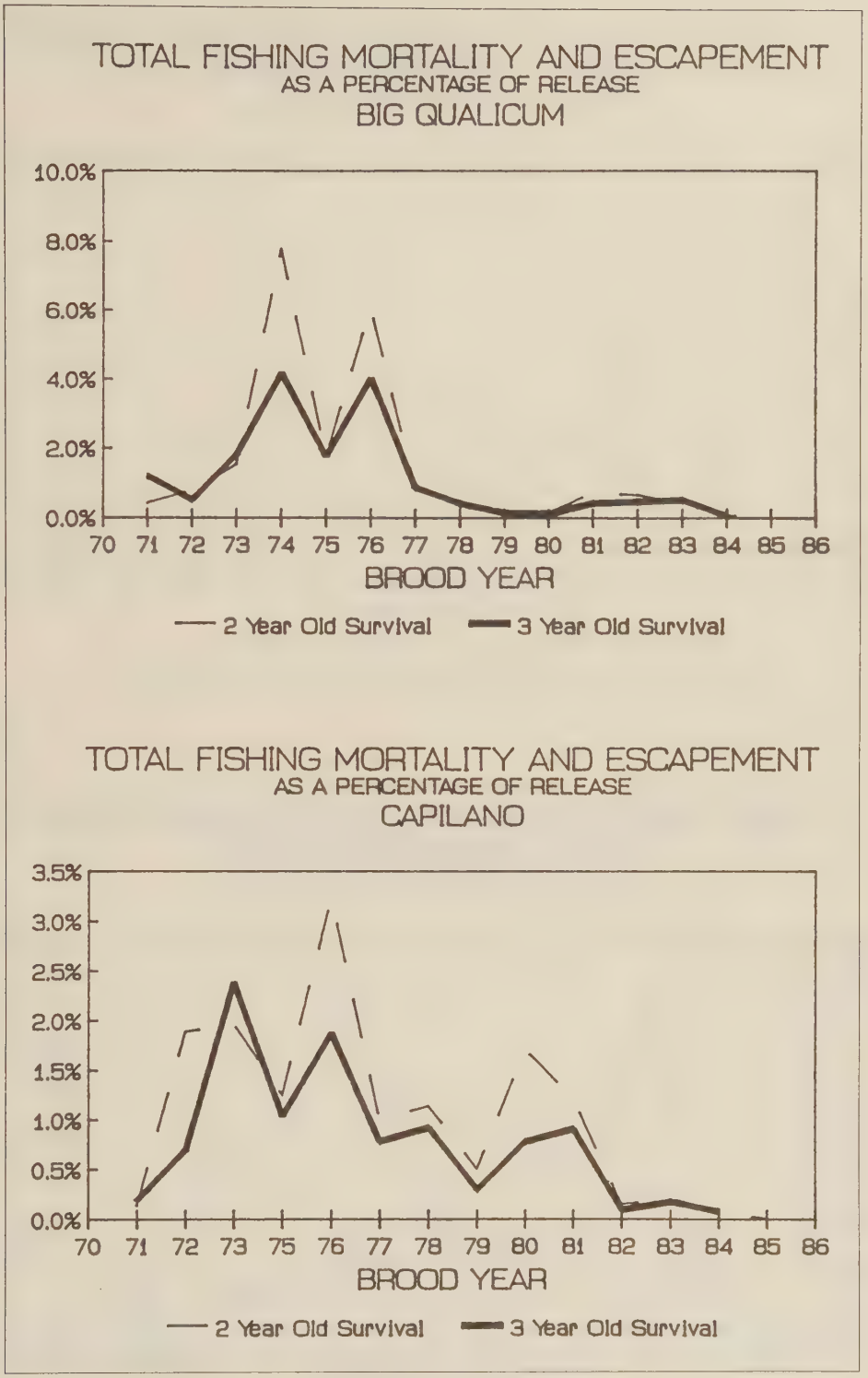
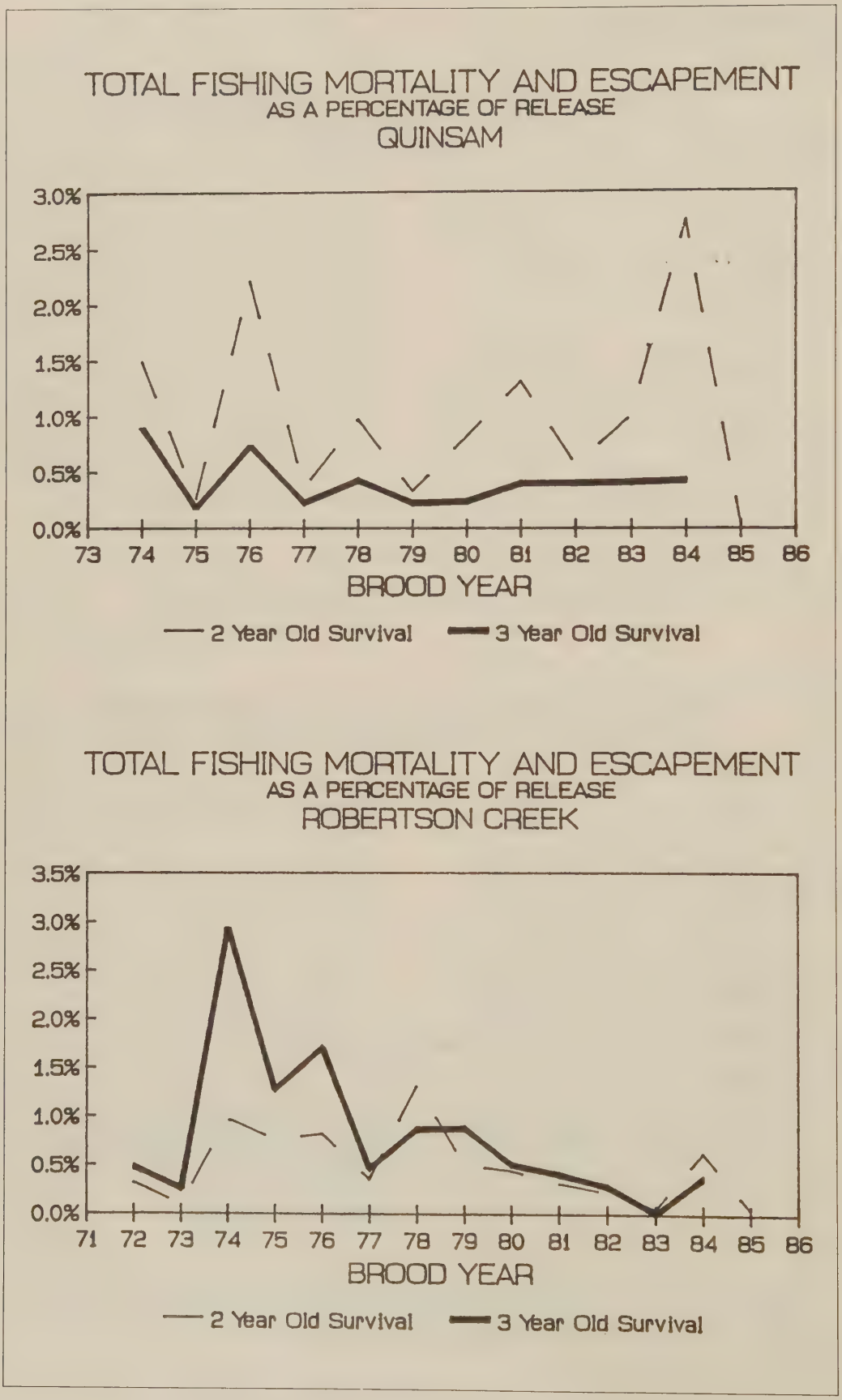
Appendix Figures



Survival rates for 2- and 3-yrs post-release for ten populations of hatchery salmon. Source: Exploitation rate analysis, Chinook Technical Committee 1987 annual report to the Pacific Salmon Commission, Vancouver, British Columbia, Canada.



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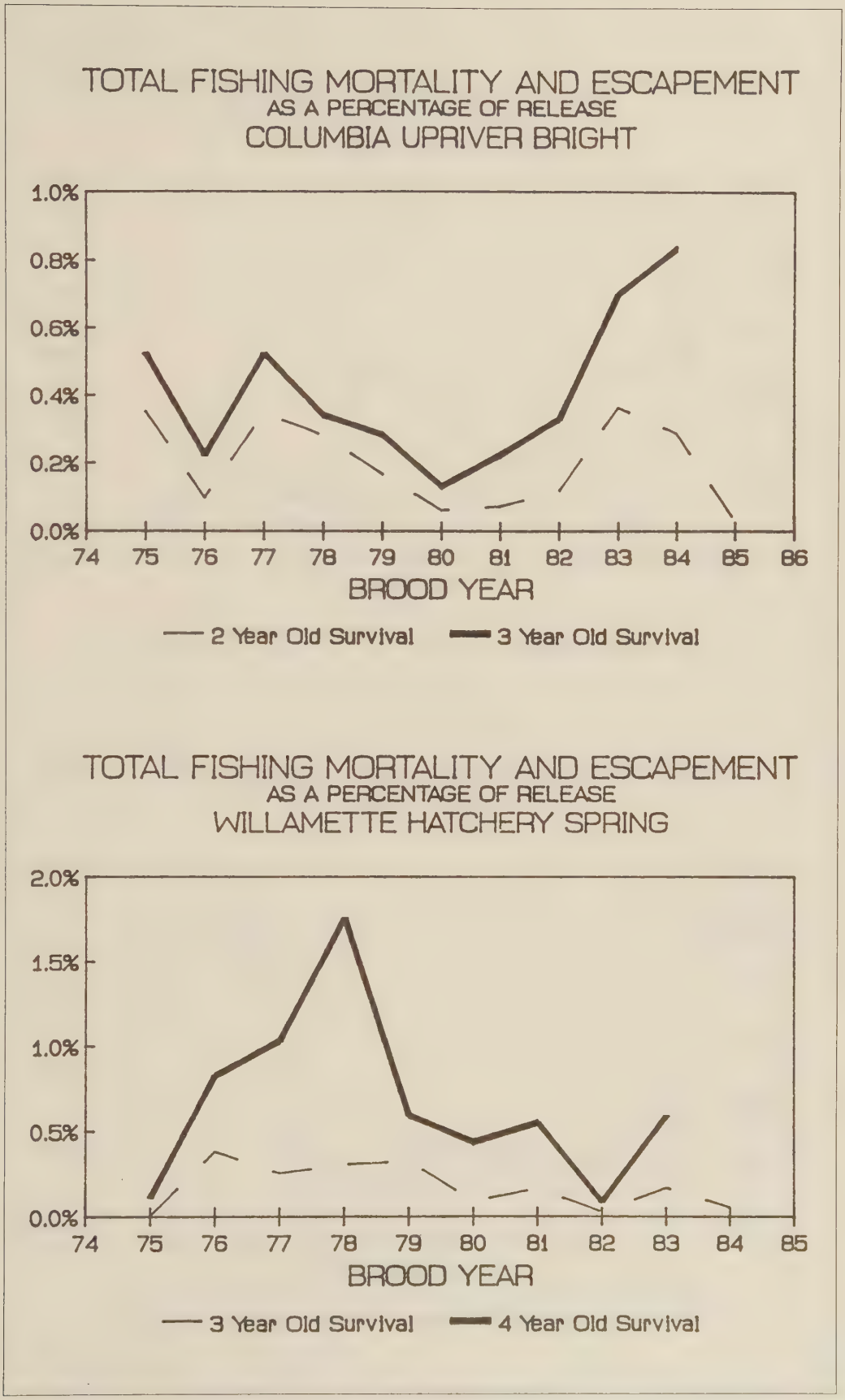

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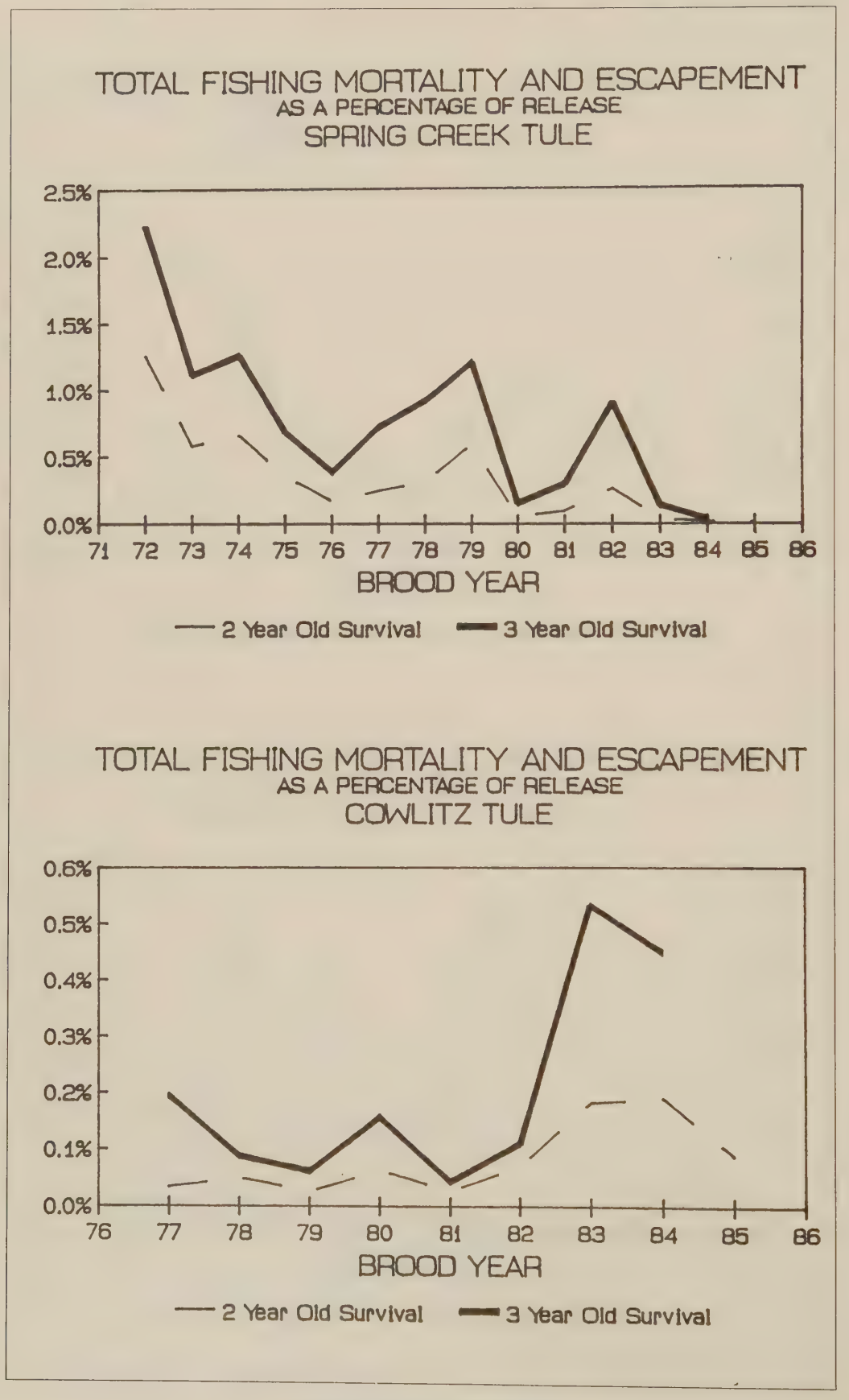

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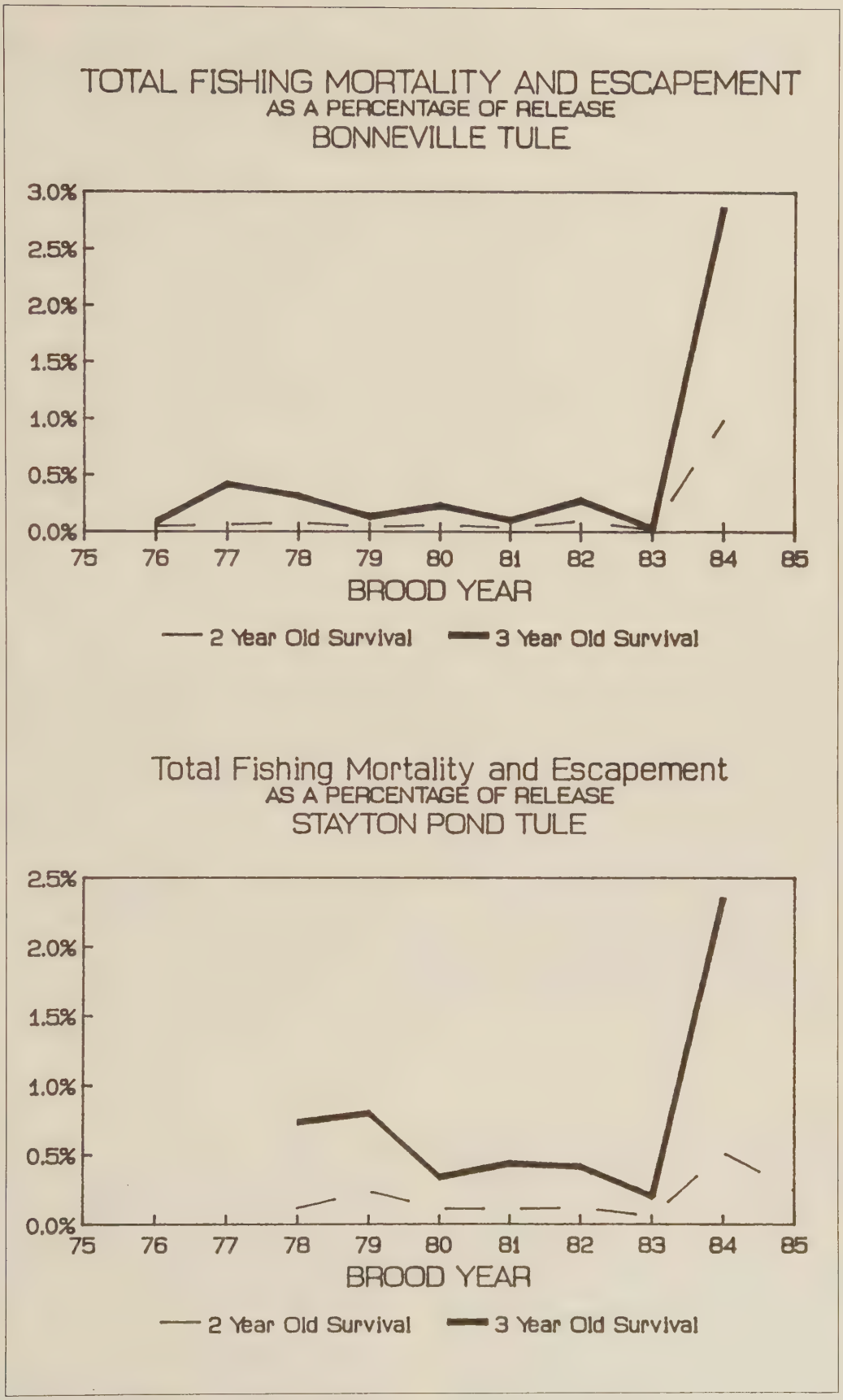

Appendix Figures (continued)

Artificial Stock-Size Improvement of the Flounder *Paralichthys olivaceus*: Present Status of Technological Achievement

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ABSTRACT

In Japan, the number of hirame (*Paralichthys olivaceus*) seed released has increased rapidly in the last decade. In 1986, about six million hirame seeds were released. The early life history of hirame and some of the technology for the release of small seeds (30–80 mm in total length) were studied in order to raise stock levels of released hirame. It was assumed that the presence of mysids as prey in the area of seed release was more critical for the survival of the seeds than for the wild hirame of the same size. Therefore, the time and area of release should be determined with reference to the seasonal change of the prey density. Acclimatization of seed to the conditions of the natural environment is one of the most important areas of study as we seek to raise stock levels of released hirame. The survival rate of reared hirame during the days immediately following their release was shown to be lower than that of wild hirame of the same size. The time off the bottom for feeding was demonstrated to be a criterion for acclimatization. Melanism, which inevitably occurs on the blind side of mass produced seeds, can be profitably noted to discriminate the released hirame from wild ones. The percentage of the commercial catch attributable to the released seed was estimated by checking the anomalies in the body color of landed hirame at the market.

Introduction

Hirame (*Paralichthys olivaceus* (Temminck et Schlegel)), a carnivorous flat fish with a maximum total length of about one meter, is one of the most important species of the Japanese coastal fishery. Male hirame usually mature in their third spring, whereas females mature in their fourth spring. The mature hirame is considered to be situated at the higher niche among the coastal demersal fishes. The production of hirame is about 7,000 metric tons a year in Japan. However, indications of overfishing in the hirame fishery have been revealed in recent years.

Mass production of hirame seeds was accomplished for the first time in 1965 (Harada et al. 1966). Then, in the late 1970's, natural spawning from brood stocks reared in tanks was achieved, and fertilized eggs of good quality became available regularly on a large scale (Hiramoto and Kobayashi 1979; Ohthuka et al. 1980). Consequently, the numer of the seeds produced for release increased rapidly,

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Table 1
Number of seeds released in Japan (thousand seeds). (Fisheries Agency and Japan Sea-Farming Association 1980–1988.

Year	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986
Number	260	297	898	2,370	1,156	2,923	3,278	4,581	4,621	5,860

exceeding twelve million in 1986 (Fisheries Agency and Japan Sea-Farming Association 1988). The number of seeds released also increased, but this is a phenomenon that has occurred only in recent years as shown in Table 1. Studies for artificial stock size improvement of hirame are centered on the technology used for the release of the seeds.

We have been carrying out the ecological studies of 0-group (i.e., juveniles less than 1-year old) hirame and their prey in the Marine Ranching Project since 1982. In this same project we also have been working to improve the technology for the release of seeds. It should be noted that prefectural fish farming centers and fisheries experimental stations supported by the Fisheries Agency have been studying technology for the release of seeds since 1980.

This report discusses the early life history of 0-group hirame and some of the technological achievements relating to the release of seeds.

Early Life History of Hirame

Hirame is a multiple spawner. The spawning season occurs when the water temperature rises to about 11°C, and it continues for 2 months (Kobayashi 1974; Odagiri 1985a). They spawn from January through August along the Japanese archipelago (Minami 1983; Odagiri 1985a). Hatched larvae live a planktonic life for a month or more (Minami 1984). In the latter half of this time period they metamorphose and immigrate into the nursery ground, the area shallower than 10 m (Koshiishi et al. 1985) or 20 m (Kiyono and Hamanaka 1974). 0-group hirame immigrate periodically into the nursery (Imabayashi 1980a; Tanaka 1988). After settling on the nursery ground, they live for about 2 months in their nursery. During their nursery life, 0-group hirame grow from 15 mm to 100–120 mm in total length. The distribution of 0-group hirame, which have grown to this size, gradually expands offshore (Kato 1985; Koshiishi et al. 1985). Afterwards, as the seasons change, they exhibit depth migration. Usually their recruitment occurs one or two years after hatching.

The food habit of 0-group hirame in the nursery is rather simple. Mysids are very important prey for them, especially in the first half of their nursery life (Imabayashi 1980b; Kato 1985; Koshiishi et al. 1985). The food composition of the 0-group hirame caught in the nursery off Igarashi-Hama (Fig. 1) is shown in Figure 2 (Koshiishi

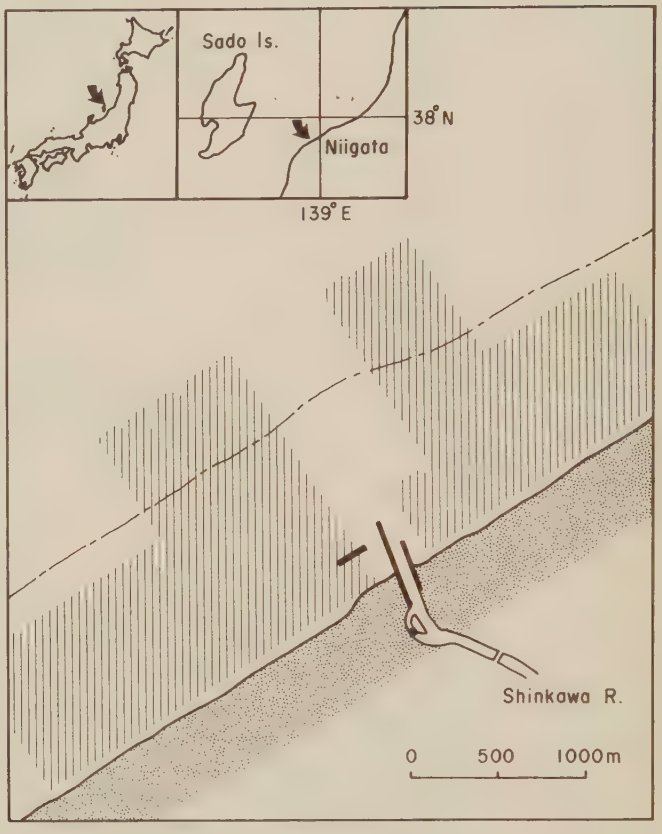

Figure 1
Research area of a nursery of the 0-group hirame off Igarashi-Hama, Niigata, Japan.

1988). Fast growth, linear with respect to the body length, was observed in this nursery. In 1987, three groups of 0-group hirame that immigrated periodically into this nursery were recognized by tracing the change in their body length (Fig. 3). The growth of the first immigrated group before the expansion of their distribution was estimated at 1.64 mm/day (Koshiishi et al. 1988). A tendency for earlier immigrating fish to grow faster was shown. This tendency was also observed in Yuya Bay (Kojima et al. 1986).

The estimated change in the hirame stock in the coastal area is shown in Figure 4. The size of hirame just after the steep decline in the stock number (Fig. 4, wild fish, time 2) may be a theoretical one for release (i.e., minimum size that can expect a relatively high survival rate after release). Takahashi (1974) estimated this size as being 50

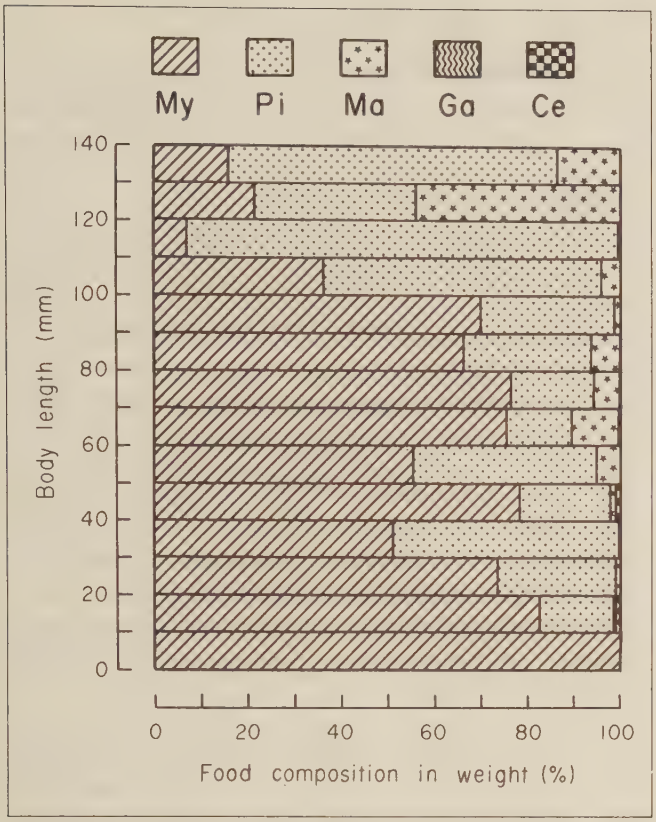

Figure 2
Food composition of the 0-group hirame of each size class (1981–1986, off Igarashi-Hama, Koshiishi 1988). My: Mysidacea; Pi: Pisces; Ma: Macrura; Ga: Gammaridea; Ce: Cephalopoda.

mm in total length, taking the changes in body proportion associated with growth into consideration. Thirty and fifty millimeters may also be the minimum sizes that correspond to the changes in feeding habit (Koshiishi, unpubl. data), and the mortality due to predation (Miniami 1986; Koshiishi 1984), respectively. The survival rate of artificially reared seeds during the early days following their release (Fig. 4, reared fish, time 2 to 3), was shown to be lower than that of wild hirame of the same size, even if their size was larger than 50 mm (Doi 1985). Therefore, raising the survival rate during the acclimatization period is one of the most important areas for raising stock levels of released hirame.

Technology for the Release of Seeds

Recently, the number of seeds released has markedly increased, reflecting technological improvements for mass production (Table 1). The size of the seeds can be divided broadly into two categories, small seeds of about 30 to 80 mm in total length, and large ones which are over 100 mm. Principally, the latter are produced to bear tags. Here we discuss the small ones.

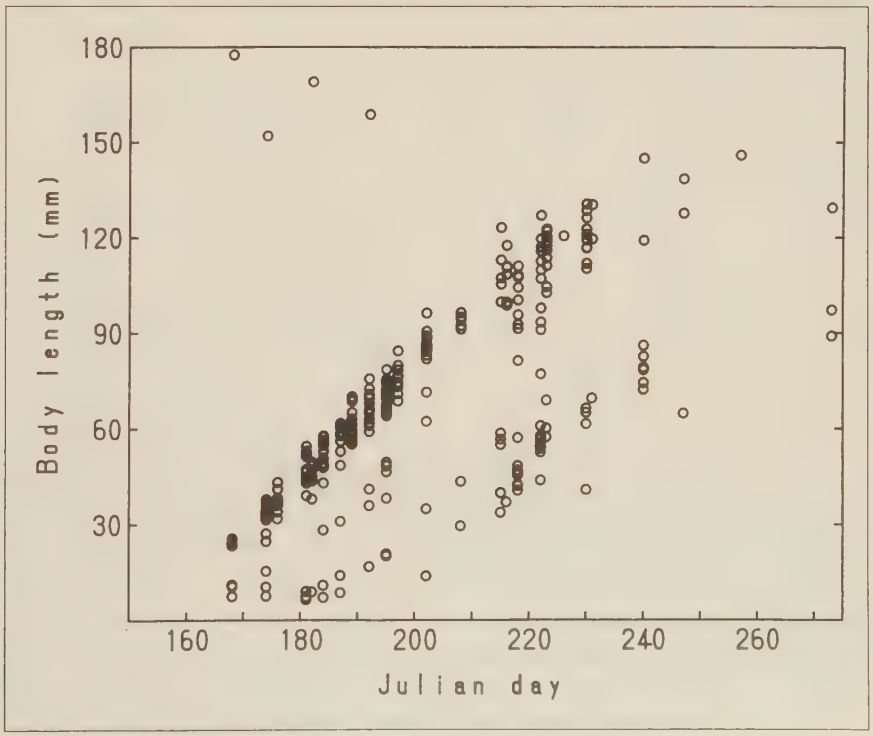

Figure 3
Change in the body length of the hirame caught in a nursery off Igarashi-Hama in 1987.

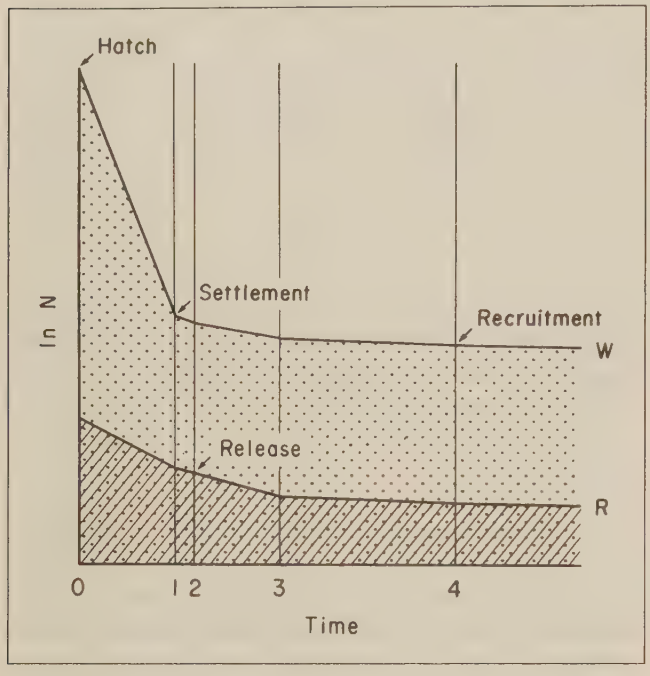

Figure 4

Schematic view of the change in hirame stock (W: Wild hirame; R: Reared hirame). Time 0 to 1 corresponds to planktonic phase (ca. 1 month). Time 2 to 3 corresponds to the acclimatization period of released seeds (ca. 2–4 weeks in the case of small size seeds 30–80 mm in total length). The survival rate of released hirame during this period is lower than that of wild ones. Usually recruitment (Time 4) occurs 1 to 2 years after hatching when their total length exceeds 25 cm or more.

Release Timing and Release Area

Our studies were carried out in the nursery off Igarashi-Hama. Basically, we focused on the relationship between 0-group hirame and their prey (mysids) distribution (Yasunaga and Koshiishi 1981).

Regarding mysids, new species are still being discovered and noted (Murano 1979), while little has been established about their ecological behavior. To catch the mysids and the other food organisms, a tow net with a rigid frame and a sledge was designed (Hirota et al. 1986, 1988). From 1984 through 1987, about five hundred tows were carried out in an area shallower than 10 m. Mysids, the most dominant group caught, separated into twenty-one species with *Acanthomysis robusta* Murano the most dominant (Hirota et al. 1988). Each species was further characterized by their seasonal and vertical abundance. Although the dominant species were most abundant at distinct depths, their abundance changed seasonally in a consistent pattern. Consequently, the number of mysids per unit area increased rapidly in spring followed by a sharp reduction in late summer (Fig. 5).

Five groups of the small size seeds (Table 2) were released from 1984 to 1987 (Koshiishi et al. 1986, 1988). They were released along a 200 m line at 4 m (1986, 1987) or 4- and 8-m (1984, 1985) depths. Flag poles with line and sinker were set up previously at both ends of these lines as a guide. The seeds were recaptured by a small beam trawl (functional mouth width: 1.7 m; mesh aperture: 2.1

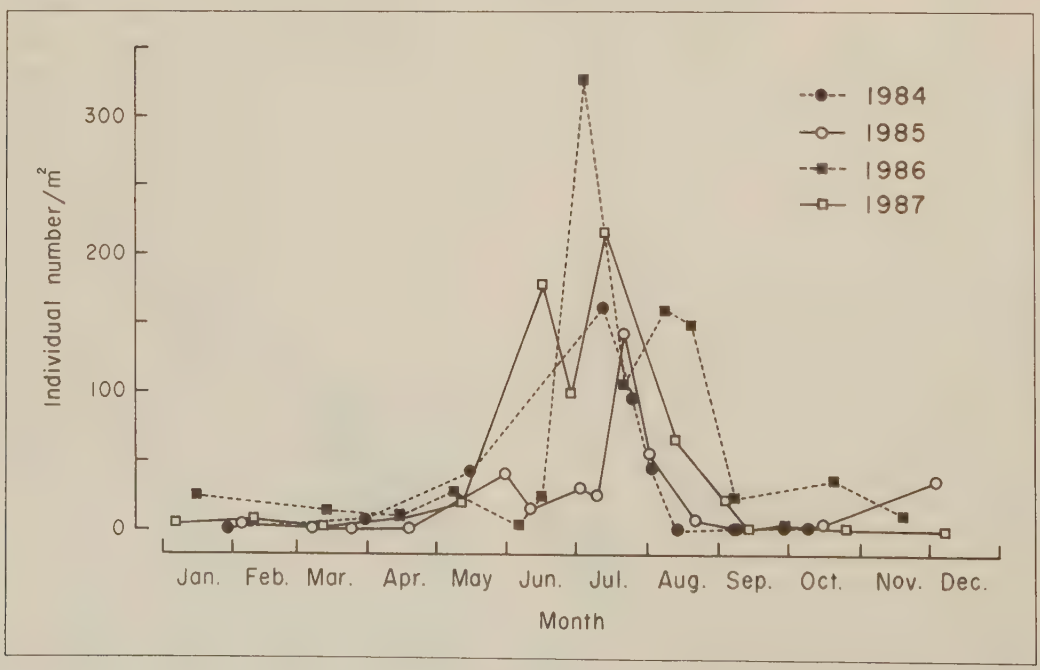

Figure 5

Seasonal change in the abundance of the mysids in a nursery of hirame off Igarashi-Hama. Mysids were caught in a depth of 2 to 10 m. (Hirota et al. 1988.)

Table 2
Date of the release, number, and total length of the seeds released in the nursery off Igarashi-Hama. (Modified from Koshiishi et al. 1986, 1988.)

Year (group)	Date	Number	Total length (mm) Mean	SD
1984	28 Aug.	26,400	57.6	10.8
1985	30 Jul.	62,100	32.5	6.3
1986	01 Aug.	50,800	37.7	—
1987N	30 Jun.	150,000	27.0	3.9
1987M	03 Aug.	52,000	30.5	3.1

or 3.6 mm), which was towed by a boat at the speed of about 0.75 m/sec. Within a month after each release, 6 to 9 days of sampling (4 to 12 hauls/day) were carried out over an area of 1 km^2, the area shallower than 10 m around the release line. The numbers of the recaptured seeds of the 1984, 1985, 1986, 1987N, and 1987M groups during this period were 30, 202, 323, 347, and 30, respectively. After weighing the whole body and measuring the total length and body length, the stomach contents of the recaptured seeds were dissected out and weighed. The feeding rate of the recaptured seeds on each sampling day was expressed as $\Sigma SCW/\Sigma BW$ (sum of stomach contents weight/sum of body weight, %). The incidence of feeding (i.e., percentage of fish that were found with food in their stomach) and the feeding rate of the recaptured seeds in 1984 and 1985 were lower than those of 1986 and 1987 (Fig. 6). Almost all the organisms ingested by the seeds

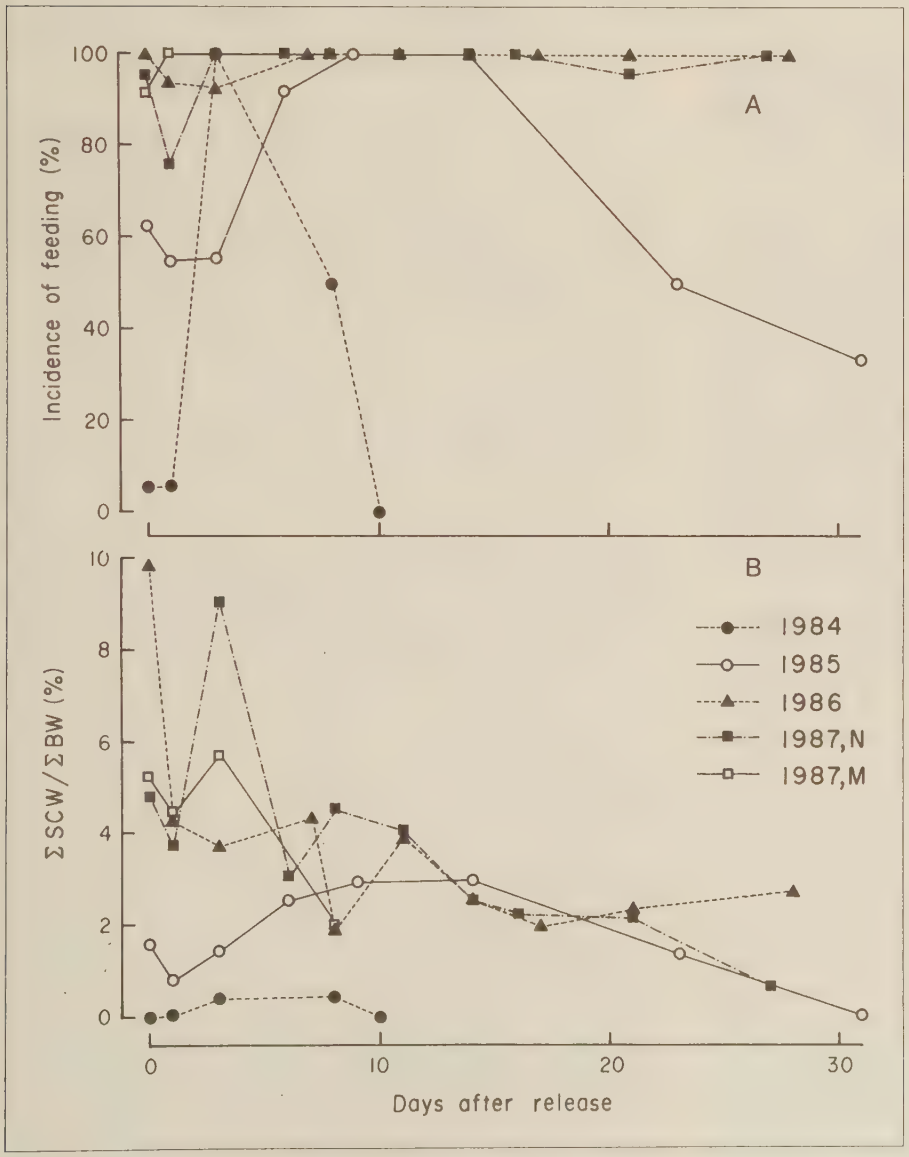

Figure 6
(**A**) Incidence of feeding (expressed as the percentage of fish found with food in their stomach) of seeds recaptured during the first month after release off Igarashi-Hama (Koshiishi 1988, modified); (**B**) feeding rate (expressed as the sum of stomach contents weight/sum of body weight, $\Sigma SCW/\Sigma BW$).

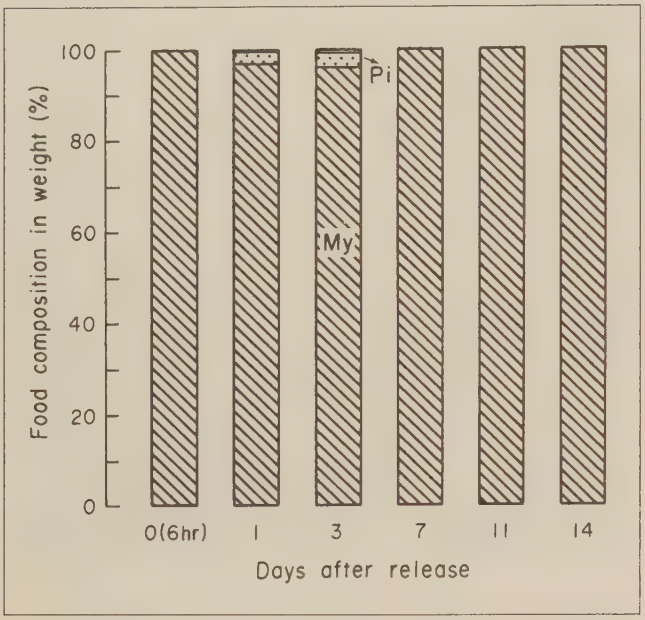

Figure 7
Food composition of the recaptured seeds during the early days after the release in 1986 (Koshiishi 1988, modified). My: Mysidacea; Pi: Pisces.

recaptured during the early days after release in 1986 were mysids (Fig. 7). This ingestion of mysids occurred during a period when high prey density existed. The feeding rate corresponded closely to the abundance of the mysids in the area where the seeds were released. In 1984, the seeds were released on 28 August, when their prey density decreased to a level near zero (*see* Fig. 5), thus the feeding rate was very low. The prey density after the release in 1985 was slightly higher but lower than that in 1986 and 1987 (*see* Table 2 and Fig. 5). No growth was observed when the feeding rate of the seeds was much lower than that of wild hirame of the same size (Table 3 and 4).

The prey densities in the research area were also reflected in the apparent survival rate during one to two months after release (Koshiishii et al. 1986; Koshiishi and Akamine 1987). Real survival rate is difficult to estimate because dispersion from the research area is immeasurable. However, from information given by fishermen concerning

Table 3
Change in the body length, incidence of feeding (INF), and feeding rate of recaptured hirame seeds during early days after release. Five groups of hirame seeds (Table 2) were released in the nursery off Igarashi-Hama. (Supplemented to Koshiishi and Akamine 1987.)

Group	Date	(Days after release)	Fish no.	Body length (mean, mm)	INF[a]	Feeding rate[b]
1984	28 Aug.	(0.21)	19	52.3	5.3	0.01
	29 Aug.	(1)	17	47.5	5.9	0.04
	31 Aug.	(3)	1	47.2	100.0	0.22
	05 Sep.	(8)	2	44.3	50.0	0.22
1985	30 Jul.	(0.25)	40	29.6	62.5	1.61
	31 Jul.	(1)	42	28.6	52.4	0.83
	02 Aug.	(3)	9	32.6	55.5	1.49
	05 Aug.	(6)	12	29.8	91.7	2.61
	08 Aug.	(9)	2	33.3	100.0	3.04
1986	01 Aug.	(0.25)	31	32.1	100.0	9.87
	02 Aug.	(1)	63	32.5	93.7	4.30
	04 Aug.	(3)	55	33.9	92.7	3.74
	08 Aug.	(7)	18	39.5	100.0	4.37
	09 Aug.	(8)	9	41.7	100.0	1.90
1987N	30 Jun.	(0.29)	22	24.4	95.5	4.82
	01 Jul.	(1)	25	22.3	76.0	3.76
	03 Jul.	(3)	23	23.5	100.0	9.07
	06 Jul.	(6)	32	26.3	100.0	3.07
	08 Jul.	(8)	23	30.3	100.0	4.53
1987M	03 Aug.	(0.25)	23	25.5	91.3	5.23
	04 Aug.	(1)	20	27.4	100.0	4.42
	06 Aug.	(3)	1	28.4	100.0	5.71
	11 Aug.	(8)	2	36.6	100.0	2.03

[a]Percentage of fish that were found with food in their stomach.
[b]ΣSCW/ΣBW(%).

Table 4
Incidence of feeding (INF) and feeding rate of wild 0-group hirame in each size class. 0-group hirame were caught from 1981 through 1987 in the shallow area off Igarashi-Hama.

Body length (mm)	10–15	–20	–25	–30	–35	–40	–45	–50	–55	–60
Fish number	40	38	35	52	52	61	57	85	63	83
INF[a]	98	100	100	100	98	97	95	92	94	92
Feeding rate[b]	3.8	4.2	4.6	4.6	5.0	3.8	3.4	2.9	2.4	2.2

[a]Percentage of fish that were found with food in their stomach.
[b]ΣSCW/ΣBW(%).

the catch of the released hirame, it seems that real survival rate also corresponds to prey density in the released area during early days after release.

Mysids were observed by SCUBA divers to be living in schools within a 5 cm layer off the bottom. Considering the feeding behavior of hirame, the availability of the mysids is estimated to be substantially high compared with other prey such as fish and shrimp. It is assumed that the presence of the mysids is more critical for the survival of released seeds than for wild hirame. This is because their ability to feed during the early days after release may be inferior to that of wild ones.

The results of the studies carried out in the nursery off Igarashi-Hama clearly indicate that the presence of a certain level of prey is necessary for good growth and thus affects the survival of the released seeds. However, the mere presence of the prey itself may not be adequate because both biotic and abiotic circumstances in the field where the seeds are released vary significantly with each case. If many predators are distributed in the release field, the prevention of predation becomes more important.

Measurement of Acclimatization

Usually the intermediate rearing of hirame is carried out in tanks on land. The rearing of demersal hirame in floating net cages at sea, as with yellowtail (*Seriola quinqueradiata*) or red sea bream (*Pagrus major*), often causes abrasion which is followed by high mortality. Therefore, rearing for mass production of seeds has been confined to tanks that are of limited capacity. SCUBA diving observations revealed that the abilities of the seeds to feed and to escape from predation were inferior to that of the wild ones (S. Furuta, Tottori Prefectural Fisheries Experimental Station, Tomari-mura Ishiwaki, Tohaku-gun, Tottori, pers. commun., May 1988). This inferiority may be caused by intensive rearing. Thus, the improvement of intermediate rearing techniques to acclimatize the seeds to their natural environment is an immediate necessity.

The establishment of a criterion to measure acclimatization is essential for the improvement of rearing methods. Physiological and biochemical measurements are useful in evaluating the activity of the seeds (Nakano and Shirahata 1988; Maruyama et al. 1986); however, in this case a criterion relating to the ecological behavior of the seeds is necessary.

Furuta (1988) estimated that the mortality of the seeds during the early days after release was caused principally by predation. Predation occurred as the seeds were swimming off the bottom to feed on live mysid. Therefore, he measured the time off the bottom of the wild and reared hirame as they fed, and established this time as a criterion for acclimatization. Furuta reared the seeds under low density condition (3 ind./m^2) and reported that the effect of this rearing on the time off the bottom was manifested after a relatively short time period (3 to 7 days). Using a net enclosure which was set on a beach to prevent the invasion of predators and which covered an area of 28,000 m^2, he also reported that rearing in the field under low density conditions was effective in raising the survival rate after release (Furuta 1988).

Thus, for acclimatizing seeds to the conditions in the field, rearing in a net enclosure can be useful and effective. However, maintenance of the net enclosure without dispersion of the seeds is fairly difficult owing to wave forces and sand drift. An effective and inexpensive facility for intermediate rearing should be planned in the near future.

Discrimination Between the Released Hirame and Wild Ones

To estimate the effect of releasing hirame, the ability to discriminate between the released and the wild ones is fundamentally important. Marking or tagging the mass produced small seeds is delicate and laborious work, and many methods have been used. For example, clipping various fins and inserting dart tags with an automatic tag applicator has been used frequently for small and large size seeds respectively. Both of these methods have shortcomings. If we clip the fin with a part of the trunk, the mark can be identified for a long time, although few are recognized by the public. Dart tags can be easily recognized, but the drop off rate is relatively high and varies with handling when inserted. Moreover, the fact that the rate of recapture was fairly low when the tags were inserted into seeds smaller than 13 cm in total length implies that the tagging is a severe encumbrance (Itano 1985).

A high percentage of anomalies in the body color has been observed in mass produced hirame seeds. These anomalies are classified into two groups: partial or full albinism on the ocular side and partial or full melanism on the blind side (Nakatani 1984). Though the causes of these anomalies are still obscure (Seikai 1985; Fukusho et al. 1986), the occurrence of the albinism on the ocular side has abated in recent years. However, the occurrence of melanism on the blind side is still high (100% in some cases). The albinic seeds are not suitable for release owing to a high mortality which seems to be caused by predation. Higher mortality of the albinic seeds than normal ones can be recognized by the steep decline in the proportion of albinic seeds recaptured after release (Fig. 8). No ecological or physiological shortcomings have been reported on melanism on the blind side yet, and the patterns of the blackish part have not changed for at least two years (Itano 1987). Melanism on the blind side has been observed among wild hirame, too, but the occurrence rate is very low, 0 to 1.0% for 0-group hirame (Odagiri 1985b), and the patterns of the blackish part seems to be different from those of mass produced seeds (Yama-

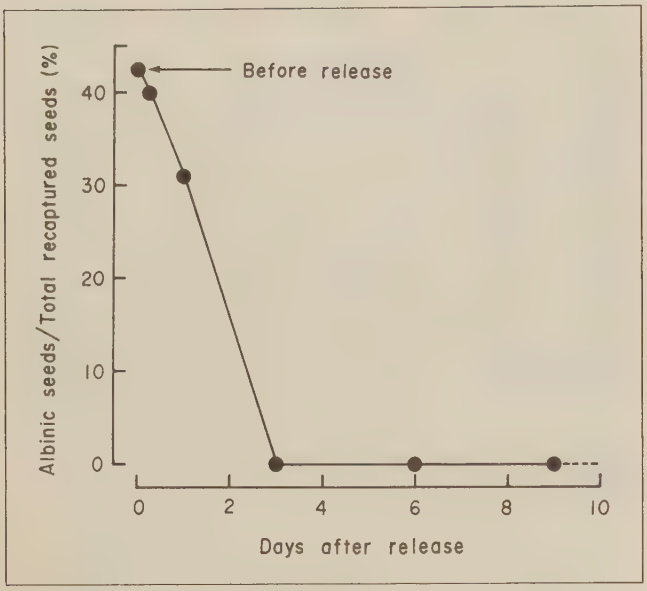

Figure 8
Change in the proportion of the albinic seeds recaptured (1985, off Igarashi-Hama). Albinic seeds: the seeds presented the symptom of albinism on more than 30% of the area of their ocular side.

Table 5
Date of the release, number, and total length of the seeds released into Mano Bay from 1980 through 1984. (Modified from Itano 1988.)

Year (group)	Date	Number[a]	Total length (mm) Mean	SD
1980	02 Jul.	35,000	25.0	—
1981	31 Jul.	47,000	38.4	7.1
1982	16 Jul.	16,500	40.8	4.9
1983	13 Jun.	69,000	33.1	6.0
1984	16 Aug.	22,200	75.4	12.1

[a]Number of the seeds without albinism on the ocular side.

moto 1985). Thus, melanism on the blind side can be profitably noted in order to discriminate the released hirame from the wild ones.

Distribution and Impact of Released Hirame Seeds Around Mano Bay

In the area around Sado Island, Niigata Prefecture, about 70 metric tons of hirame are landed every year (Itano 1982). These hirame are netted mainly in and around Mano Bay where the Niigata Prefectural Fish Farming

Figure 9
Recapture area of the seeds released into Mano Bay from 1980 through 1984. About 190,000 seeds were released from 1980 through 1984.

Center is located. The hirame population distributed around Sado Island is thought to be isolated from that of Honshu. About 190,000 seeds without albinism on the ocular side were released from 1980 through 1984 (Table 5) by the Niigata Prefectural Fish Farming Center (Itano 1988). The occurrence rate of melanism on the blind side of these seeds was high, from 80 to 100%. Before release, we asked all members of the Fisheries Cooperative Association on Sado Island to inform us of the capture of hirame with melanism on the blind side. The points of recapture extended across an area of over half way around the island (Fig. 9). More than 90% of these points were located in and around Mano Bay. The hirame with melanism on the blind side were checked out at four markets near the main fishing ground. Landed hirame were measured for their total length and their body color checked on the blind side at the markets before auction. The checkup was carried out year round, almost every day at the main market, and thus about 20% of the yearly catch was checked. The per-

Table 6

Percentage of the catch of hirame with anomalies in their body color on the blind side. Landed hirame that were caught by fishermen in and around Mano Bay were checked for body color at four markets.

Year	1984	1985
Total catch in weight (kg)	20,166	16,428
Total catch in number	42,690	39,030
Percentage of the fish with anomalies in their body color[a]	11.9	11.8

[a] 90% + of this figure may be attributable to the released hirame.

centages of the catch with anomalies in the body color were 11.9 and 11.8% in 1984 and 1985, respectively (Table 6). Ninety percent or more of these hirame with anomalies were estimated to be attributable to the release from their proportion or pattern of the blackish part on the blind side. The other hirame, which numbered less than 10%, developed only slight anomalies (e.g., those with small blackish part at their caudal fin on the blind side), and thus a discrimination between the released and the wild fish could not be made. In 1985, the age of the recaptured hirame ranged from 1 to 4 years, and the 2-year-old group accounted for nearly 70% of the recapture (Itano 1986). The large number of seeds released in 1983 (Table 5) may be reflected in the latter age composition.

Beneficial Effects of Releasing Hirame Seed

The effect of releasing hirame has been clearly demonstrated in some districts where fishermen have started the release program by themselves. However, in order to improve the stock size, many problems need to be overcome in the near future. Among these problems the following ones are fundamentally important.

The abundance of the 0-group hirame in nurseries have fluctuated markedly (Koshiishi and Akamine 1987) as shown in Table 7, and the average density has been estimated at about 100,000 ind./km^2 (Takahashi 1974). Compared with the number of the wild 0-group hirame, the number of released seeds is still too small. Therefore, the releases must continue on a regular basis in order to improve the hirame stock size.

The high market price of hirame has caused overfishing in recent years. However, little economic effect can be expected when the seeds are recaptured before they are given a chance to grow up. Therefore, management of the hirame fishery will be required in order to improve its profitability.

Table 7

Estimated number of 0-group hirame in the nurseries. (Supplemented to Koshiishi and Akamine 1987.)

Nursery	Year	Number/km^2
Taisha Bay	1983	500,000
Off Shichirinaga-Hama	1984	77,500
	1985	8,750
Off Igarashi-Hama	1984	320,000
	1985	320,000
	1986	49,000

Acknowledgments

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Reproductive Sterility in Triploid Pacific Oysters

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ABSTRACT

Triploid Pacific oysters (*Crassostrea gigas*) were produced as part of a research program at the University of Washington to alleviate problems of low marketability of oysters in the summer. The immediate aims of this investigation were to evaluate the feasibility of producing triploid oysters reliably, to determine the extent of sterility, and to scale the procedure up to commercial levels for use by the industry.

Results of experiments to induce triploidy with cytochalasin B were successful. Gametogenesis in triploids was investigated and triploidy was found to retard gametogenesis in oysters. Gonad production in triploids was reduced by 50 and 75% in males and females, respectively, as measured by the proportion of cross-sectional area of the body occupied by gonadal tissue. Histologically, females produced some eggs, but there was a wide variation among individuals in the extent of egg production. Males underwent spermatogenesis and produced spermatozoa, but this process was delayed compared with that of diploid controls. Carbohydrate reserves remained high in triploids because of their reduced reproductive effort.

Triploidy improved the marketability of oysters. Consumer taste panels and trial marketing in the Pacific Northwest area have demonstrated that triploids are marketable during the summer when the fecundity of normal diploid oysters makes them unpalatable. In contrast, product quality is maintained in the triploids because the reduced reproductive effort helps retain their solid meat texture and the higher carbohydrate levels retain their sweet taste.

Triploids have been embraced by the industry. Commercial hatcheries in the Pacific Northwest are currently producing them for both the half-shell and shucked meat trade. Triploid oysters represent about 10% of the production for two major companies in Washington. Production at one company supplied approximately 80,000 gallons of shucked triploid oysters in 1988 and, at the other, approximately 10,000 dozen were produced for the half-shell trade. Both hatcheries project that a greater proportion of their production will be triploids in the future.

Two outstanding and unexpected features of gametogenesis in triploids are described below, but future investigations will need to be done to determine the "whys." First, there are a high proportion of hermaphrodites among triploids. Analysis of gametogenesis was performed on three populations. One population of triploids was produced by mass spawning and, after setting, were grown out in Humboldt Bay, California. Two other populations of triploids were produced as half-sib families (one male fertilized the eggs from two females to produce families 306 and 251) Both of these families were planted in Mud, Oakland, and Rocky Bays, all located in southern Puget Sound.

*Present location: Rutgers Shellfish Research Laboratory, P.O. Box 687, Port Norris, NJ 08349.

In the diploid control oysters from Humboldt Bay, 1 of 78 was hermaphroditic, while among the triploids (siblings of the diploids), 29 of 101 were hermaphroditic. Similarly, summing over all three bays in Puget Sound, families 306 and 251 had no hermaphrodites among the diploid controls sampled (sample size: 15 and 36, respectively) and among the triploids, families 306 and 251 had 24 hermaphrodites of 49 sampled and 12 hermaphrodites of 27 sampled, respectively. In Mud, Oakland, and Rocky Bays the respective incidence of hermaphrodites was a) 44, 35, and 69% for Family 306 and b) 27, 50, and 50% for Family 251. The incidence of hermaphrodites in triploids may have a genetic basis.

The second unexpected feature was the production of haploid sperm by triploid male Pacific oysters. Most of the spermatozoa that are produced by triploids are 1.5N or aneuploid as judged by flow cytometric analysis of gametes. However, many males also produce 1N or true haploid gametes in higher proportions than would be expected from a binomial distribution based on random segregation of the third chromosome set. Production of haploid sperm within an individual was not uniform throughout the gonad: different regions of the gonad of one individual produced 1N or 1.5N sperm or both. Families were produced from backcrossing five triploid males to diploid females and resulted in metamorphosis and setting in four of the five families. All of the surviving offspring were diploid. The cytogenetic mechanism for the production of 1n sperm in triploid males is unknown.

Marine Afforestation of *Eisenia bicyclis* (Laminariaceae; Phaeophyta)

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ABSTRACT

The structural variations within communities of sublittoral marine algae were investigated in the Pacific coast of northeastern Honshu, Japan. Fundamental data were obtained to support the creation of a marine afforestation of *Eisenia bicyclis* in an area presently covered by crustose coralline red algae considered a "sea desert." The algal community was usually segregated by depth into three zones populated predominantly by either *E. bicyclis* (0–7 m), *Dilophus okamurai* (7–8 m), or crustose corallines (8–9 m). Variation in seawater temperature in the months from January to July affected the population dynamics of these and other species in waters 2–7 m in depth. When the average water temperature was low, the zones occupied by both *E. bicyclis* and *D. okamurai* changed as increased numbers of juvenile *E. bicyclis* appeared. Similarly, the quick growing *Undaria pinnatifida* became established in the zone predominanted by *D. okamurai*. The following year, a dominant population of *E. bicyclis* resulted as the previous year's juveniles developed. When the average water temperature was high, densities of the large brown algae of both *E. bicyclis* and *U. pinnatifida* decreased, and a population of *D. okamurai* became dominant. Feeding by herbivorous predators were abundant in the crustose corallines and *E. bicyclis* subzones, but few in the *D. okamurai* subzone. The thallus of *D. okamurai* was found to have spatane-type diterpenes which may have deterred predation by herbivorous animals. Afforestations of several algae were established in a sea desert area for two years in order to create an environment suitable for culture of the abalone *Haliotis discus hannai*. Concrete blocks fitted with three kinds of seeded string having approximately 0.1-mm-long young sporophytes of either *E. bicyclis*, *U. pinnatifida*, or *Laminaria japonica* were placed in 3- to 7-m-deep water over a 1,000 m^2 wide area, to form a forest dominated by *E. bicyclis*. The following year, 8,000 juvenile abalone, approximately 5.3 cm in shell length, were released into the artificially created forest. Two years later, up to 62.4% of the abalone were recaptured and well grown (approx. 9.1-cm shell length).

Introduction

Communities dominated by the large perennial brown alga, *Eisenia bicyclis*, with stipe as long as 1 m or more, are the most typical marine forests along the Pacific coast of Honshu, Japan. Annual net production of the *E. bicyclis* population was estimated to be 20 kg wet weight/m^2/year (Yoshida 1970), equivalent to the highest level of annual net production of the terrestrial forests in the temperate zone (Yokohama et al. 1987). In some areas off the Pacific coast of northeastern Honshu, *E. bicyclis* populations often disappear, and dense growth of crustose coralline red algae becomes established covering all rock surfaces. Such a phenomenon, called "Isoyake" in Japanese or "sea desert" in English, are supposed to occur because of the following factors: 1) competition for substratum with other algae (Iwahashi et al. 1979); 2) overgrazing by herbivorous animals (Nakahisa 1980); 3) hydrographic changes (Kawajiri et al. 1981); 4) decline of transparency and increase in sediments by polluted sea water (Yokohama 1982). However, it can be said that the conclusive factor responsible for the formation of the sea desert has yet to be clearly established.

The need to develop the technology to create artificial marine forests of *E. bicyclis* has become pressing because of its use as a feed for commercially valuable abalones and sea urchins.

In this study the structural variations in the algal communities inhabited by *E. bicyclis* were observed along the Pacific coast of northeastern Honshu. After collecting

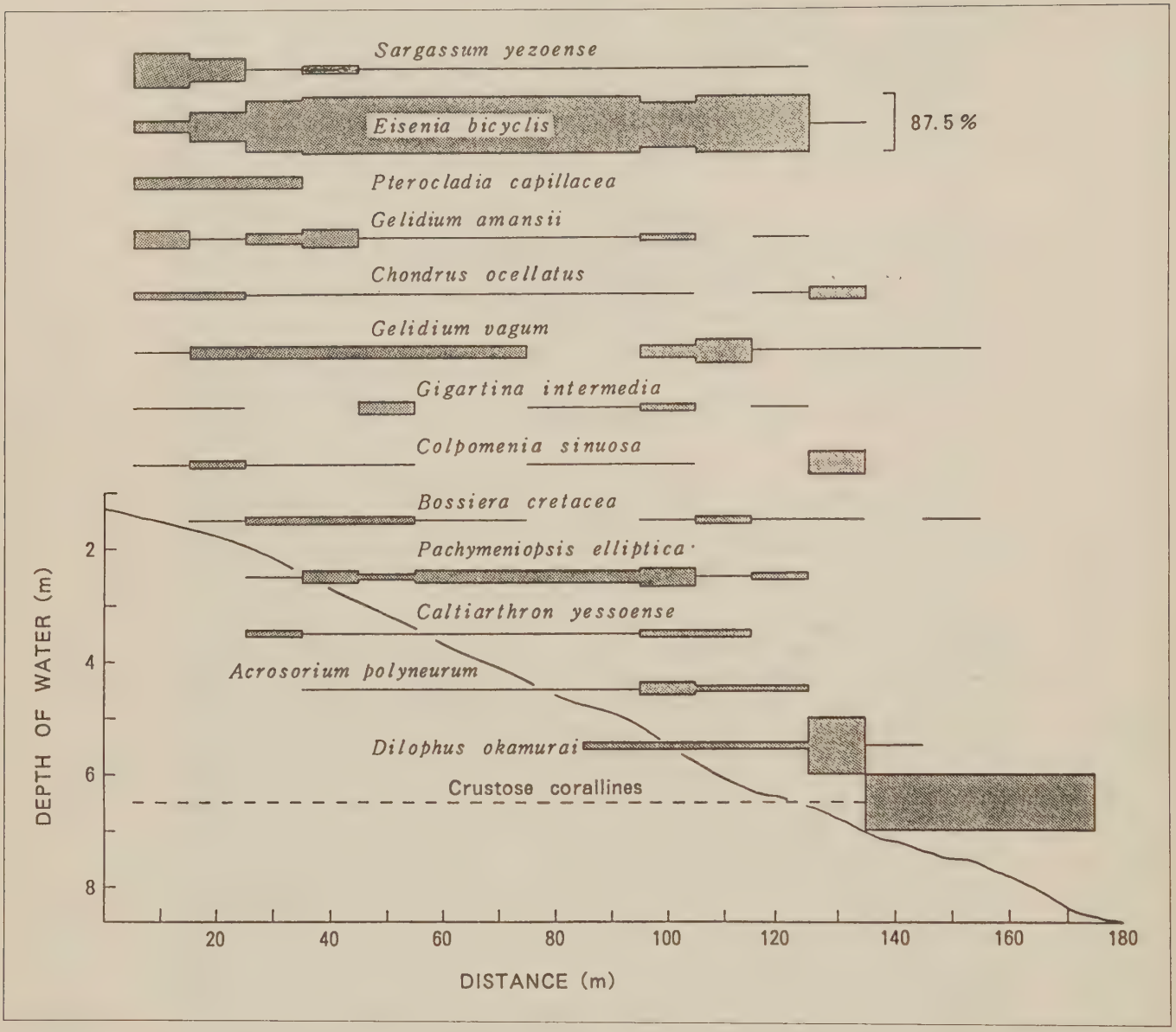

Figure 1
Distributional pattern of major benthic algae coverage with the bottom topography of the coast of Oshika Peninsula, June 1987.

fundamental data on this area of the sublittoral zone, marine afforestation of *E. bicyclis* began on a sea desert area at Matsushima Bay, Miyagi Prefecture. In this experimental area, many juvenile abalone, some tagged, were released and their growth observed after two years.

Structural Variation in the *Eisenia bicyclis* Population

Off the coast of the Oshika Peninsula, in Miyagi Prefecture, algal communities on the sublittoral rock slope can usually be classifed into three groups according to the dominant species (Fig. 1). The large perennial brown alga, *E. bicyclis*, is predominantly located in the shallow subzone between 0 and 7 m in depth, along with small perennial red algae, such as *Pterocladia capillacea*, *Gelidium amansii*, *G. vagum*, and *Pachymeniopsis elliptica*. The small annual brown alga, *Dilophus okamurai*, occurs in the middle subzone between 7 and 8 m and crostose coralline red algae in the area below 8 m. This structure of algal communities is very common along the Pacific coast of northeastern Honshu.

Figure 2 shows the proportions of herbivorous animals in the three algal communities. In the *E. bicyclis* community, several species of herbivorous predators were found. The sea urchin, *Strongylocentrotus nudus*, occupies 84.4% of

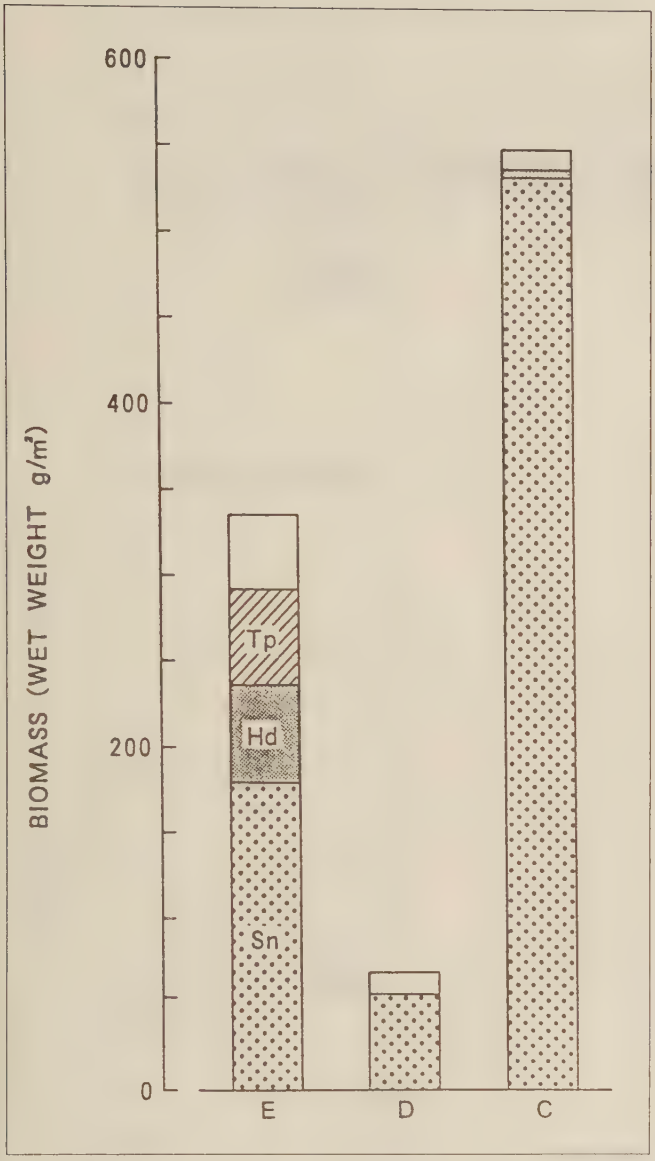

Figure 2
Mean standing crop (g/m^2) of herbivorous animal communities dominated by *Eisenia bicyclis* (E), *Dilophus okamurai* (D), and crustose coralline red algae (C) off the coast of Oshika Peninsula, sampled June 1987. Values were obatined by six quadrats (2 × 2 m) settled in each community. Tp = *Tegula pfeifferi*; Hd = *Haliotis discus hannai*; Sn = *Strongylocentrotus nudus*; blank = others.

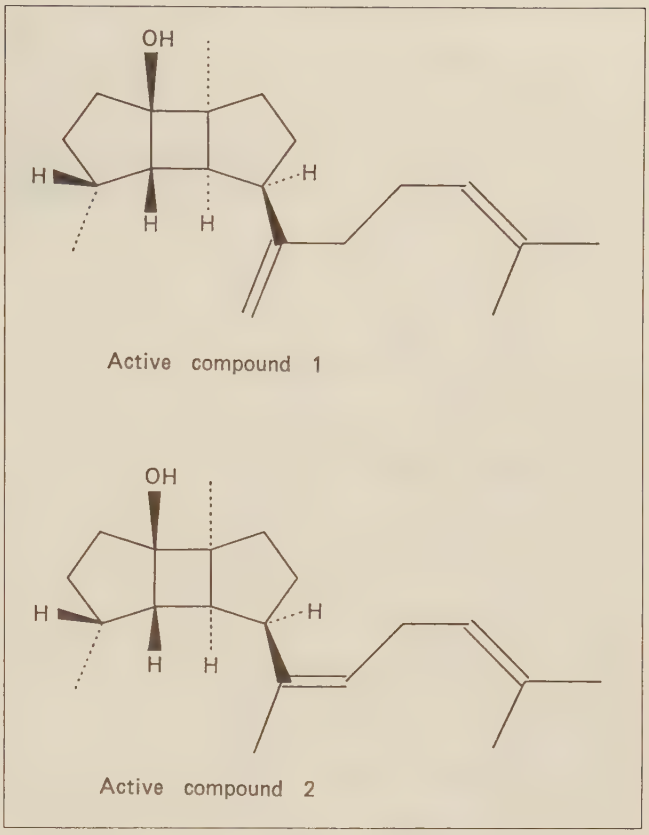

Figure 3
Structures of two active spatane-type diterpene alcohols isolated from the thallus of *Dilophus okamurai*.

the *D. okamurai* zone and 97.1% of the crustose coralline community.

During the present survey, we found that the *D. okamurai* community contained few predators. This suggested that perhaps the thallus of *D. okamurai* contains toxins which deter predation. A bioassay of the neutral fraction of a methanol extract from the thallus induced abnormal behavior, mutation, and death of the veliger of the abalone, *Haliotis discus hannai* (Taniguchi et al. 1989), as well as antifeeding activities for juvenile *H. discus hannai* and *S. nudus*. Two active spatane-type diterpene alcohols (Fig. 3) were isolated with repeated bioassays and purifications of the neutral fraction (Kurata et al. 1988).

Observations on the structural variations of the algal communities were first made off the Joban coast, in Fukushima Prefecture from 1980 through 1982 (Fig. 4, Taniguchi et al. 1986). During these three years, *E. bicyclis* remained dominant and stable in the area less than 2 m in depth. In the deeper area of 2–6 m, the dominant species varied by year (i.e., the donimant species was *D. okamurai* in 1980, *Undaria pinnatifida* in 1981, and *E. bicyclis* in 1982). The *E. bicyclis* population included plants of various ages (Fig. 5). High recruitment of *E. bicyclis* in 1981 and a dominant population of one-year-old plants in 1982 was observed. In 1981, seawater temperatures during the germination period of *E. bicyclis* from January to July, which were caused by the First Oyashio Branch, were conspicuously low (Fig. 6).

The close relationship between seawater temperature and the occurrence of the various algal species was also observed off the coast of Oshika Peninsula (Fig. 7, Taniguchi et al. 1987). In 1983, *E. bicyclis* showed low recruit-

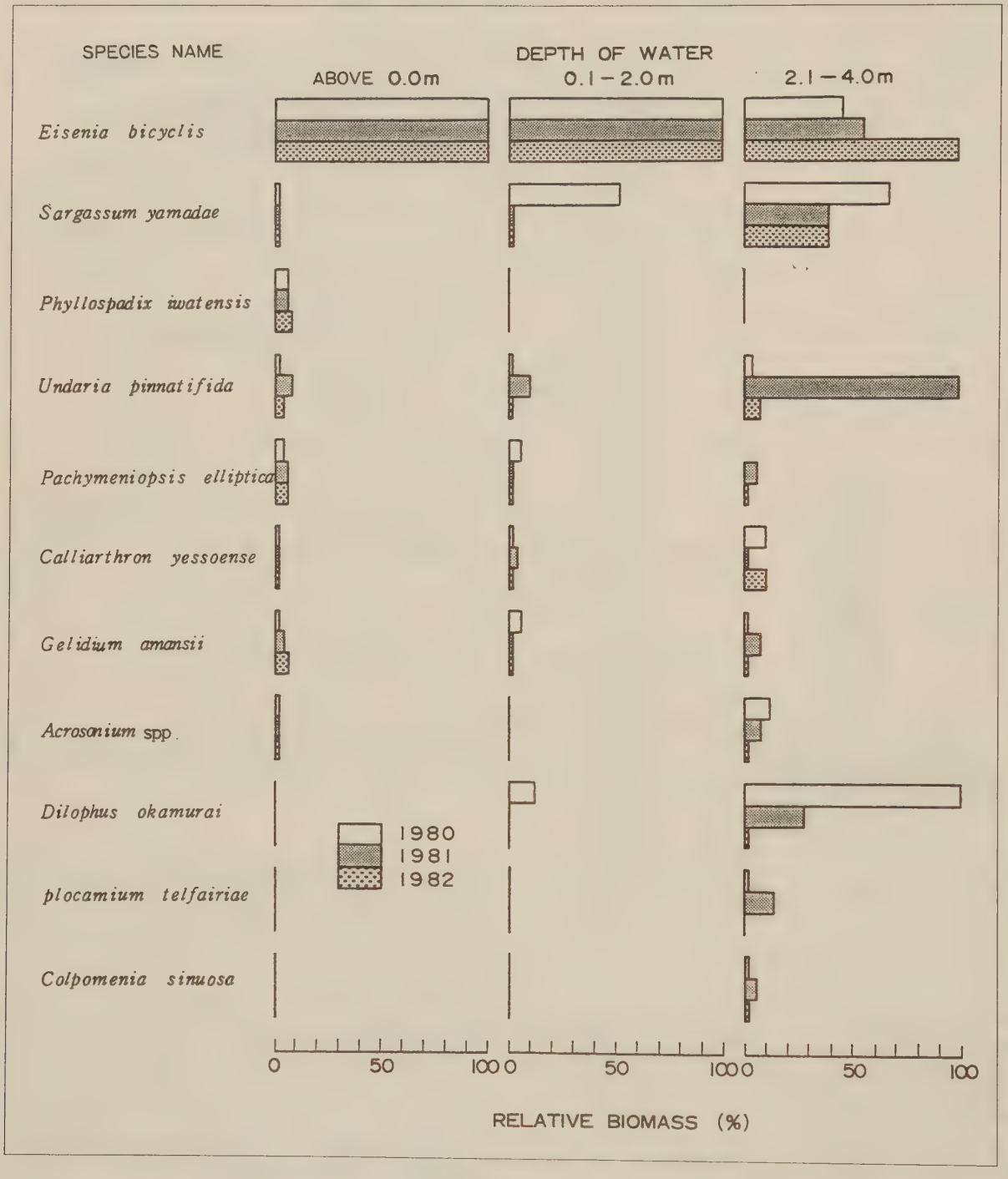

Figure 4
Yearly changes of major benthic algae biomass (%) in the three depth ranges in Joban coast, from 1981 through 1982.

ment and the population decreased. In this year, large annual brown algae, such as *U. pinnatifida*, *Laminaria japonica*, and *Costaria costata*, decreased and *D. okamurai* increased, conspicuously.

Thus far the results suggested that *E. bicyclis* remains dominant in shallower areas, stabilized by varously aged plants and by its high density. However, the population in the lower limit area of 6–8 m is unstable and sensitive to high temperatures experienced during the germination period.

It is suggested that the phenomenon known as sea desert, may be a result of the crustose coralline community be-

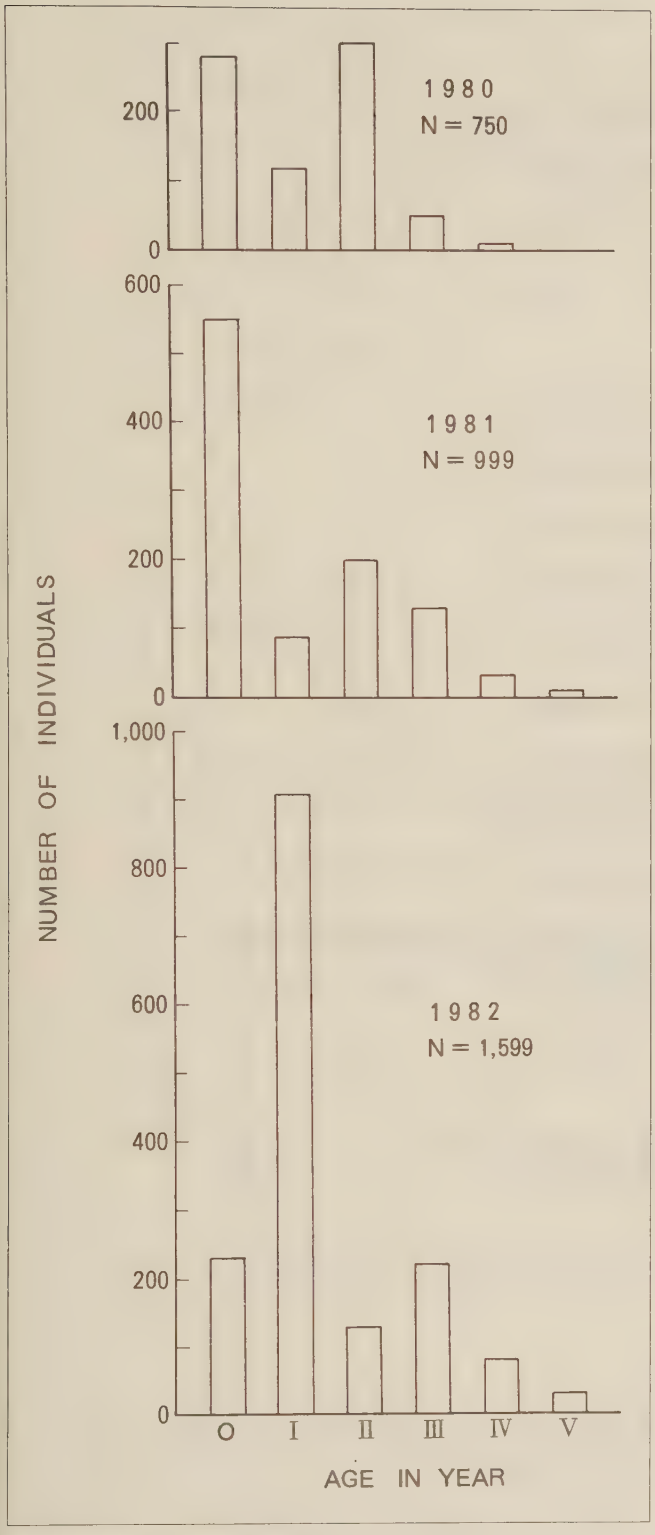

Figure 5
Age distribution of *Eisenia bicyclis* population along the Joban coast, from 1980 through 1982.

coming dominant during changes in the algal communities' structure and maintained by the high grazing pressure of herbivorous animals.

Age Composition of *Eisenia bicyclis* Population

Eisenia bicyclis populations are composed of 0- to 6-year-old plants (Taniguchi and Kato 1984). The composition was examined in the populations located within two permanent quadrats off the coast of Oshika Peninsula, from August 1983 through December 1986 (Fig. 8, Taniguchi and Kito 1988). Variations in the relationship between recruitment and extinction by grazing, withering, or detachment were examined. The population density of newly germinated plants was largely governed by seawater temperature from January to July; the rate of recruitment was conspicuously high during periods of low seawater temperature but low at high temperatures. In general, the plants which germinated at low water temperatures maintained the dominant population. The annual rate of mortality for existing plants was estimated to be 70% in the first year, 60% in the second year, 40 to 50% for plants 2-4 year old, and 80% for the 5-6 year old population.

The newly germinated plants decrease rapidly in areas with a higher density of adult plants probably on account of low light intensities caused by the canopy of the adult plants. If the older adult plants in the population are low in density, the young plants survive well. The *E. bicyclis* community includes individuals from each age group that serve to adequately maintain the populations over time.

Establishment of Artificial Reefs

The experimental establishment of artificial reefs was carried out for five years in a sea desert area between 1 and 8 m in depth off Funairi-jima Island at the mouth of Matsuchima Bay (Fig. 9). The area is rocky, composed of boulders and cobblestones. Seawater temperatures ranged from 23.3°C in August to 5.0°C in February during the years from 1984 to 1988.

During preliminary investigations in July 1983, 35 species of algae were recorded. At a depth more than 1 m, large algae had low standing crops below 100 g/m^2. The sea urchin *S. nudus* was the most dominant herbivorous animal, with an average standing crop of 144 g/m^2. In August 1982, 10 reefs were artificially constructed for the marine afforestation of *E. bicyclis* at a depth of 3 m at an inlet to Funairi-jima Island. Seeded strings with approximately 0.1-mm-long young sporophytes of this alga were transplanted to the reef in December 1982. However, the juveniles of this alga disappeared completely in September 1983, because they were devoured by herbivourous animals.

Seventy-three more artificial reefs, fastened by ropes to each other were set at 3–7 m in depth in an approximately 1,000-m^2-wide area in August 1983. The three kinds of seeded string of either *E. bicyclis*, *U. pinnatifida*,

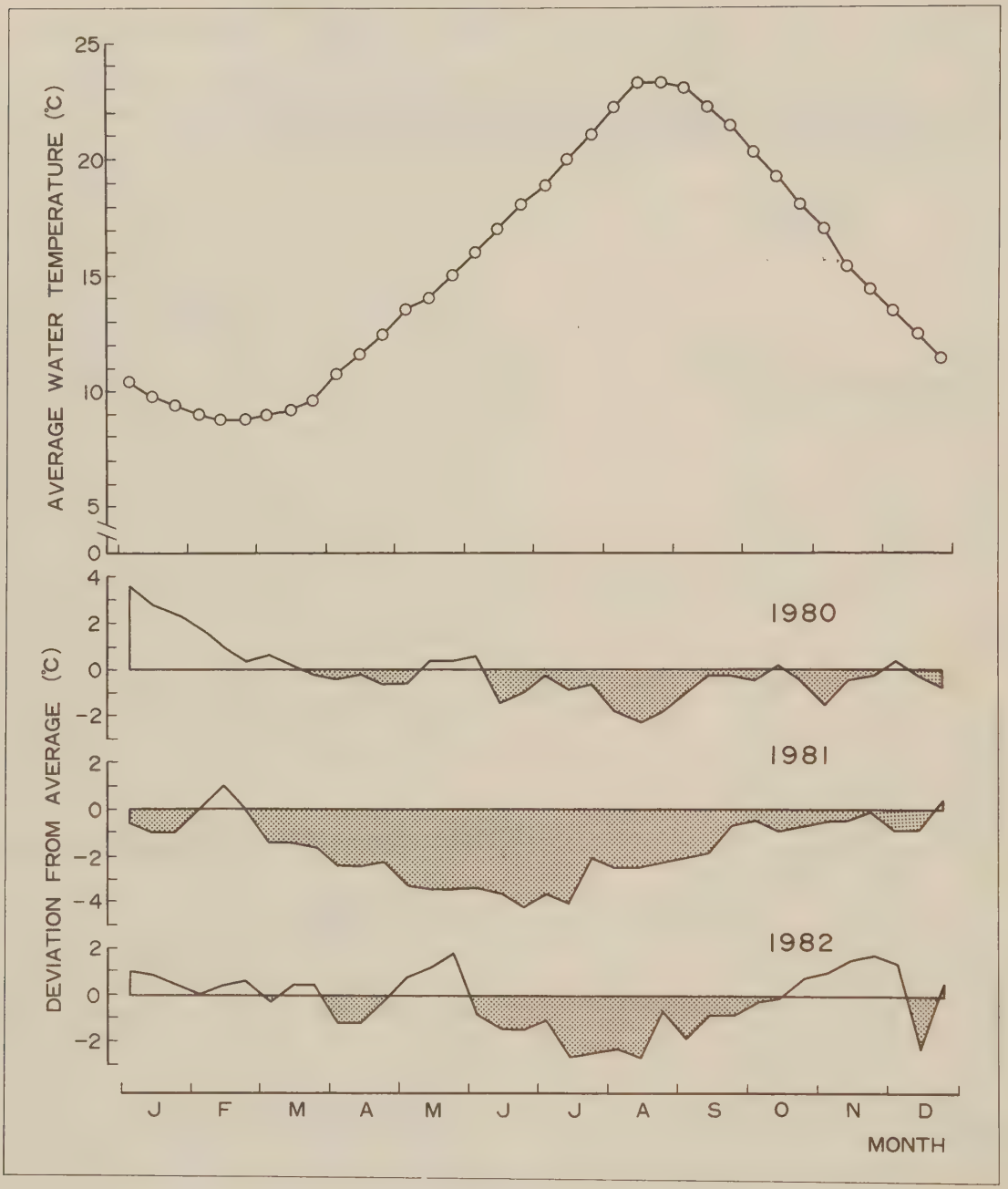

Figure 6

Seasonal variation of the sea surface temperature along the Joban coast, obtained from ten-day mean values during the period from 1941 through 1970 (**upper panel**), and ten-day mean surface temperatures (deviations from the value in the upper panel) for 1980 through 1982 (**lower panels**).

or *L. japonica* were attached to the artificial reffs in December 1983. The seeded string of *L. japonica* was transplanted to the ropes.

Undaria pinnatifida grew rapidly and occupied the surface of the artificial reefs three months after seeded string transplantation. *Laminaria japonica* occupied the surface after six months. From December 1983 to September 1984 the total production of *U. pinnatifida* and *L. japonica* was estimated at approximately 6.7 tons from each 438 m of seeded string on the artificial reefs. The *L. japonica* biomass was approximately 2 tons from 60 m of culture line.

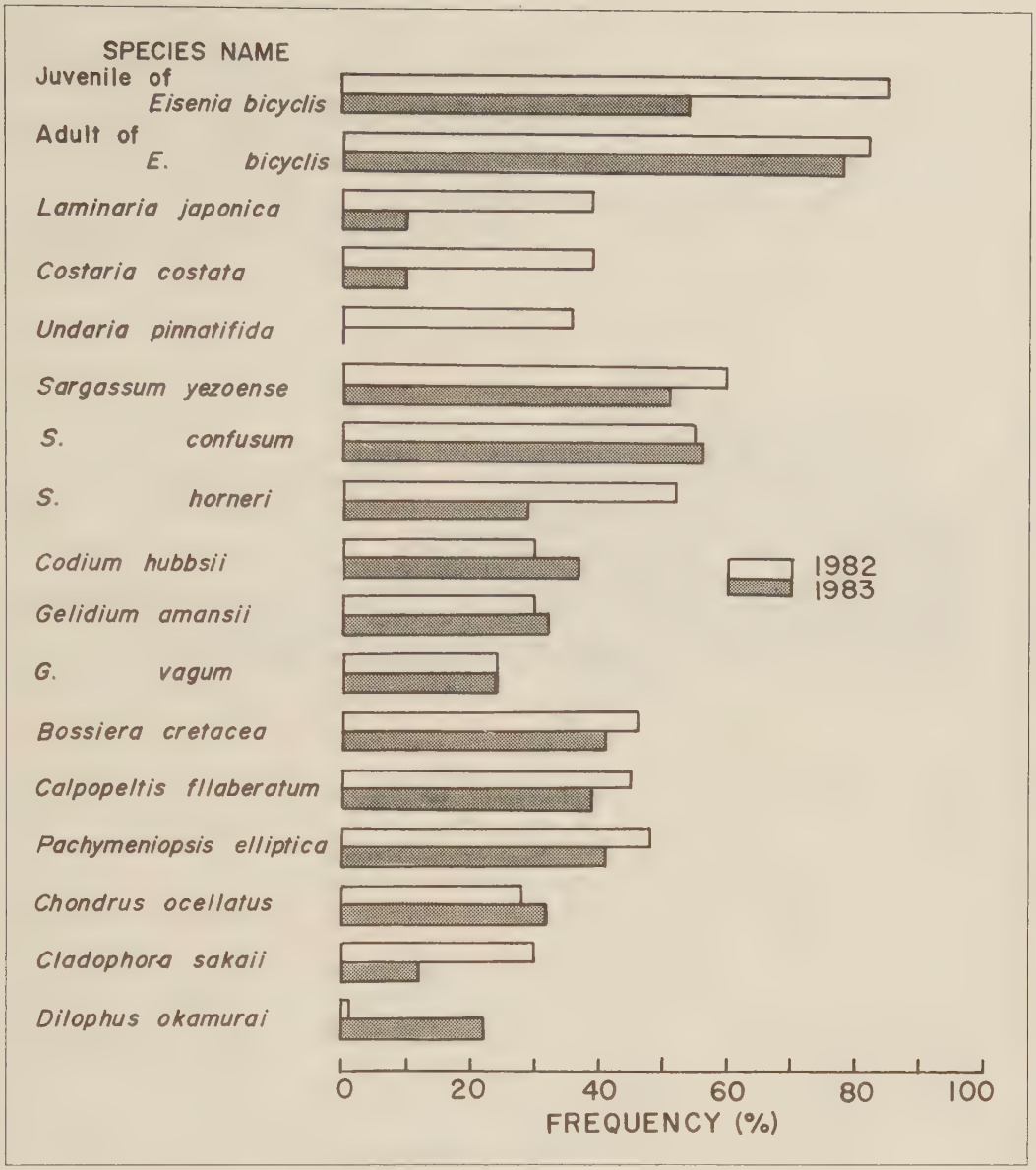

Figure 7
Number of quadrats (%) in which major benthic algae appeared in the survey area off the coast of the Oshika Peninsula in July 1982 and August 1983.

Eleven months later, from December 1983, *E. bicyclis* occupied the surface of the artificial reefs. By that time *U. pinnatifida* and *L. japonica* had decreased. The population densities of the transplanted *E. bicyclis* and the newly regenerated populations after 1986 are shown in Figure 10. The newly regenerated populations were also observed on the sea bottom. The combined *E. bicyclis* biomass from plants on the artificial reef and the sea bottom for the years 1984 through 1987 were 1-9, 1.1, 3.0, and 2.3 tons, respectively. However, the population densities were low compared with average density of 10–20 plants/m^2 in natural populations (Taniguchi et al. 1987). This may be due to the fact that 1) seawater temperatures were high during the years from 1986 to 1988; 2) the biomass of herbivorous animals gradually increased reaching high levels before transplantation of seaweeds; and 3) approximately 8,000 abalone were released in July 1986, further increasing the effects of predation (discussed below).

Production of Abalone in the Artificial Reefs

In order to assess the productivity of abalone in an afforestation area, juvenile abalone, *H. discus hannai*, measuring 53.5 ± 4.8 mm in shell length and 24.4 ± 2.2 g in body

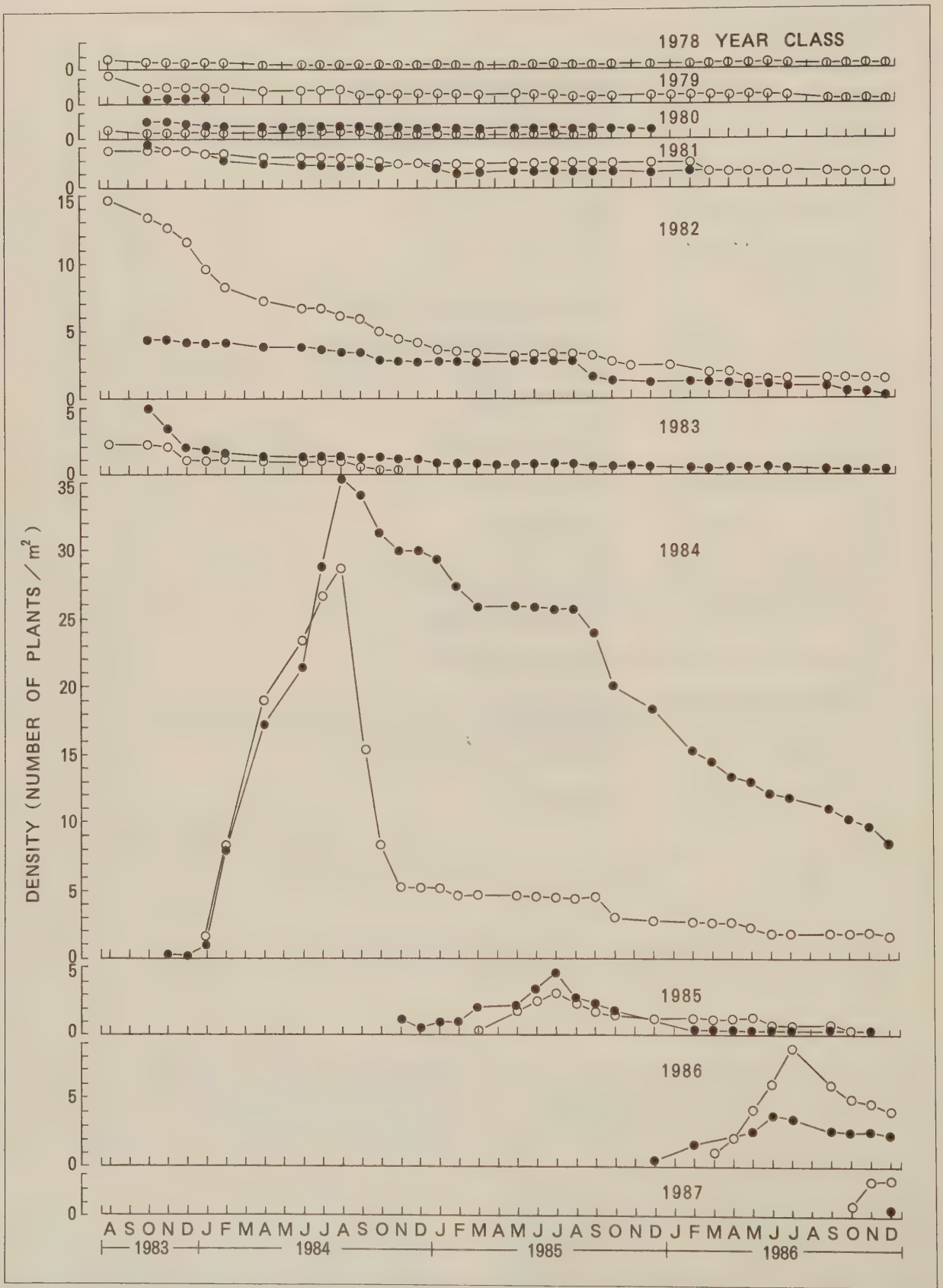

Figure 8

Seasonal variation of density (number of plants/m^2) of various year classes of *Eisenia bicyclis* at station 1 (open circles) and station 2 (solid circles) along the coast of Oshika Peninsula from August 1983 through December 1986.

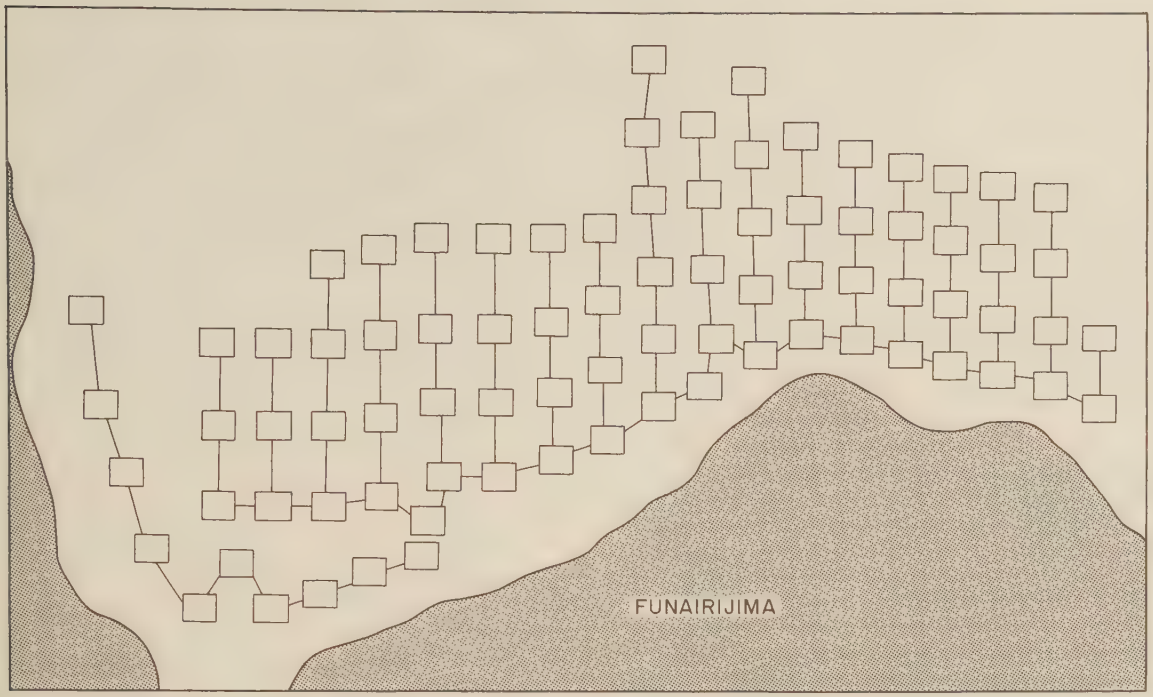

Figure 9
Artificial reefs that were fastened to each other by ropes, designed for marine afforestation of *Eisenia bicyclis*.

Figure 10 (below)
Seasonal variation of *Eisenia bicyclis* density (number of plants/m^2) at the experimental marine afforestation station in Matsushima Bay from July 1984 through July 1988.

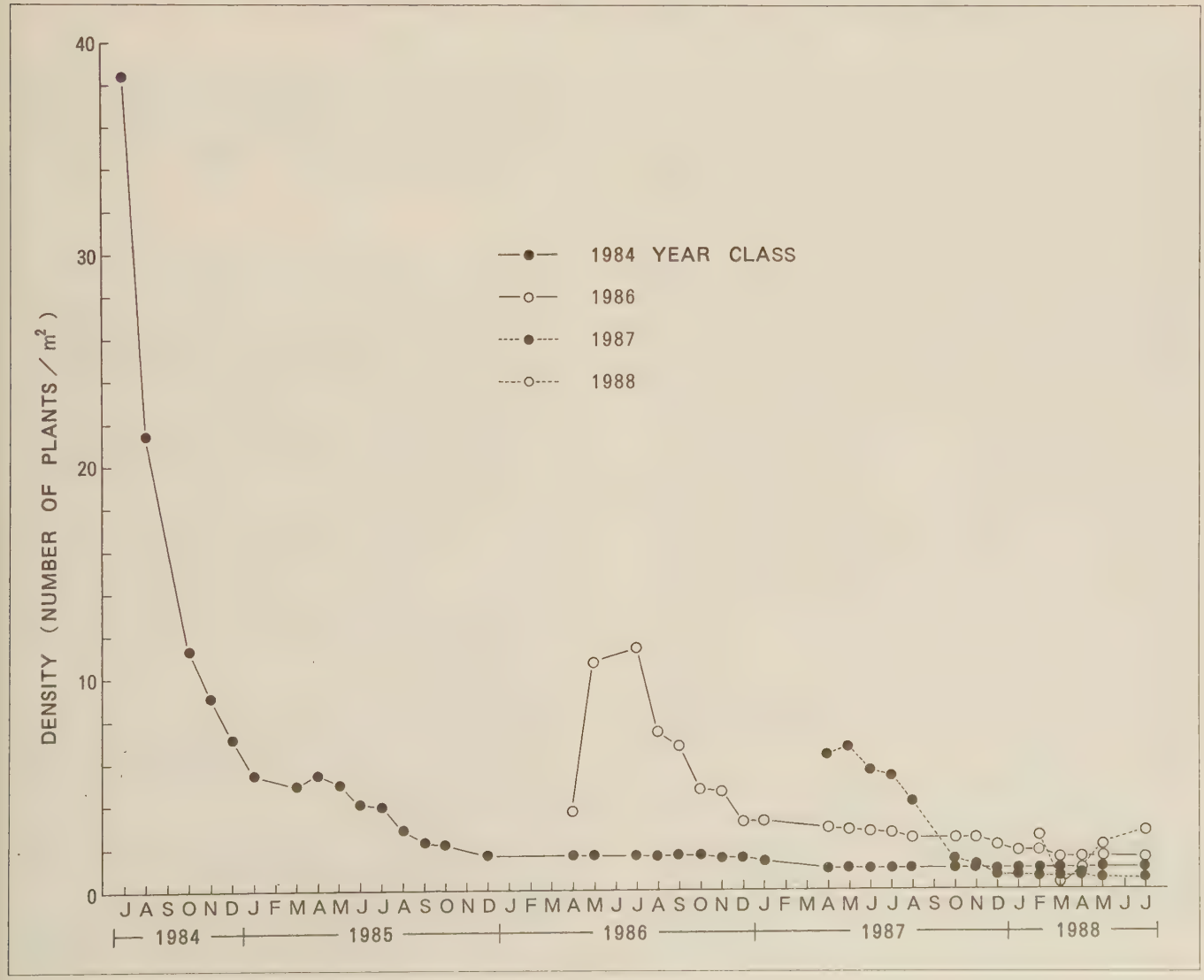
DENSITY (NUMBER OF PLANTS / m²)
40
30
20
10
0
1984 YEAR CLASS
1986
1987
1988
J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J
1984
1985
1986
1987
1988

Table 1

Recapture record of reared abalone two years after release and estimation of the standing stock (Taniguchi and Yamada 1987).

	Afforested area	Natural field-I	Natural field-II
Square of cultured field (ha)	ca. 1	ca. 1	ca. 0.4
Number of released abalone	8000	8000	3000
Total number of abalone caught (T)	2579	3879	1841
Shell length (mm)[a]	93.3 ± 9.7	100.6 ± 8.9	74.7 ± 10.2
Body weight (g)[a]	111.9 ± 39.0	152.7 ± 43.6	67.9 ± 31.7
Recapture number of released abalone (r)	2379	2334	916
Shell length (cm)[a]	91.6 ± 9.3	97.3 ± 7.9	67.3 ± 7.9
Body weight (g)[a]	11.9 ± 33.7	134.8 ± 31.8	48.2 ± 17.9
r/T (%)	94.3	83.0	55.2
Estimation of standing stock size			
Number of tagged abalone	100	100	117
Recapture rate of tagged abalone (%)	45.0	55.0	62.4
Standing stock size estimated	5344	5713	2863
Survival rate of released abalone (%)	82.0	76.1	72.8

[a]The values were obtained from a 10% sampler.

weight were transplanted into three experimental areas: 1) an afforestation area; 2) a high primary production area with *E. bicyclis* and *Sargassum horneri* (natural field I), and 3) 3000 into a sea desert area (natural field II) in Matsushima Bay (Taniguchi and Yamada 1988). Recapture data after two years is shown in Table 1. In an afforestation area, 2579 abalone totalling 357 kg in weight were caught by fishing. The standing stock size at the start of the fishing was estimated at 5344 individuals totally 771 kg in weight by Petersen's method. Released abalone accounted for 94.3% of the catch, measuring 91.6 ± 9.3 mm in shell length and 111.9 ± 33.7 g in body weight. The rates of growth in the released abalone were approximately 19.9 ± 7.1 mm/year and 43.8 ± 17.8 g/year with 82.0% survival in an afforestation area, 23.5 ± 6.3 mm/year and 55.2 ± 22.5 g/year with 76.1% survival in Natural field I, and 6.8 ± 1.1 mm/year and 12.9 ± 12.8 g/year with 72.8% survival in Natural field II. The growth rate compares favorably with the values obtained under excellent natural field conditions, and the survival rate was much higher than that in most natural fields.

As of July 1988, only three adult plants/m^2 of *E. bicyclis* were found. It is believed that these plants survived because *U. pinnatifida* or *L. japonica* were grazed by predators instead of *E. bicyclis*.

The results of this marine afforestation experiment suggest that change from a crustose coralline community to an *E. bicyclis* community can be achieved artificially under the proper environmental conditions. Predation and seawater temperatures during the *E. bicyclis* germination period are major biological factors influencing the success of such a venture. It was further verified that this man-made control over the sea desert environment is an effective technology for the practical management of abalone and sea urchin production.

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Salmonid Carrying Capacity: Estimates and Experiences in the Great Lakes of North America

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ABSTRACT

During the first half of this century, a long and complicated history of overexploitation of fisheries and invasion by exotic species left the Laurentian Great Lakes with depauperate and low-value fish communities. Beginning in the 1960's, introduction of hatchery-reared exotic salmonids created a recreational fishery estimated to have an economic return of $3–4 billion per year. Ten years ago we used energetics models and expected stocking rates to estimate the total predatory demand. We forecast a predator-induced decline in the dominant forage species. More recently, primary prey species have declined in Lakes Michigan, Huron, and Superior. Maintenance and allocation of the forage base is now a major concern for management agencies. Effective management of all the Great Lakes fisheries now requires the consideration of salmonid stocking effects on the food web in each lake. Principles and practices derived from the Great Lakes may be useful in developing management policies for other systems where predator populations are enhanced by stocking.

Introduction

Enhancement of desirable fish stocks through hatchery production and stocking has a long and somewhat checkered history. Stocking is widely practised in the management of inland waters and is, in many cases, the major if not sole support of fish populations. This paper reviews the experiences derived from large scale salmonid stocking as a management practice employed in developing fisheries of the Laurentian Great Lakes of North America. Although these systems are smaller and less complex than the marine environments where salmon enhancement programs are practised, some guidance may be derived from the lessons and tools employed in developing management strategies for large lakes.

Fish communities and fisheries of the Great Lakes (Superior, Michigan, Huron, Erie, and Ontario) have been in constant flux since the early part of this century (Kitchell and Crowder 1986). Invasions by exotic species, notably sea lamprey (*Petromyzon marinus*) and alewife (*Alosa pseudoharengus*), coupled with an intense fishery exploitation resulted in the decline of most native piscivores. Local extinctions were common. With few piscivores present, alewife became the dominant planktivore, replacing a suite of native species—including several endemic ciscoes (*Coregonus* spp.)—that had served as the forage base for native piscivores and that had supported major commercial fisheries. Alewife populations burgeoned to the extent that massive die-offs were common and resulted in major economic costs because of clogged water intakes and fouled beaches (Eck and Wells 1987).

Sea lamprey control measures, first implemented in the 1950's, allowed reintroduction of top predators such as the native lake trout (*Salvelinus namaycush*) (Kitchell and Crowder 1986). During the 1960's, management agencies began stocking Pacific salmonids such as coho (*Oncorhynchus kisutch*) and chinook salmon (*O. tshawytscha*), rainbow trout (*O. mykiss*) and the European brown trout (*Salmo trutta*) in an attempt to create a biological control for the overly abundant alewife (Scavia et al. 1986). In addition, pink salmon (*O. gorbuscha*) were accidently introduced to Lake Superior and subsequently invaded each of the lakes. Unlike the other salmonids that are maintained largely or only by annual stocking, pink salmon have established self-sustaining populations through natural reproduction.

While management through salmonid stocking was first and most intensively developed for Lake Michigan, similar practices have since evolved in each of the five lakes. The assemblage of stocked salmonids has been very effective in controlling alewife abundance and now supports a sport

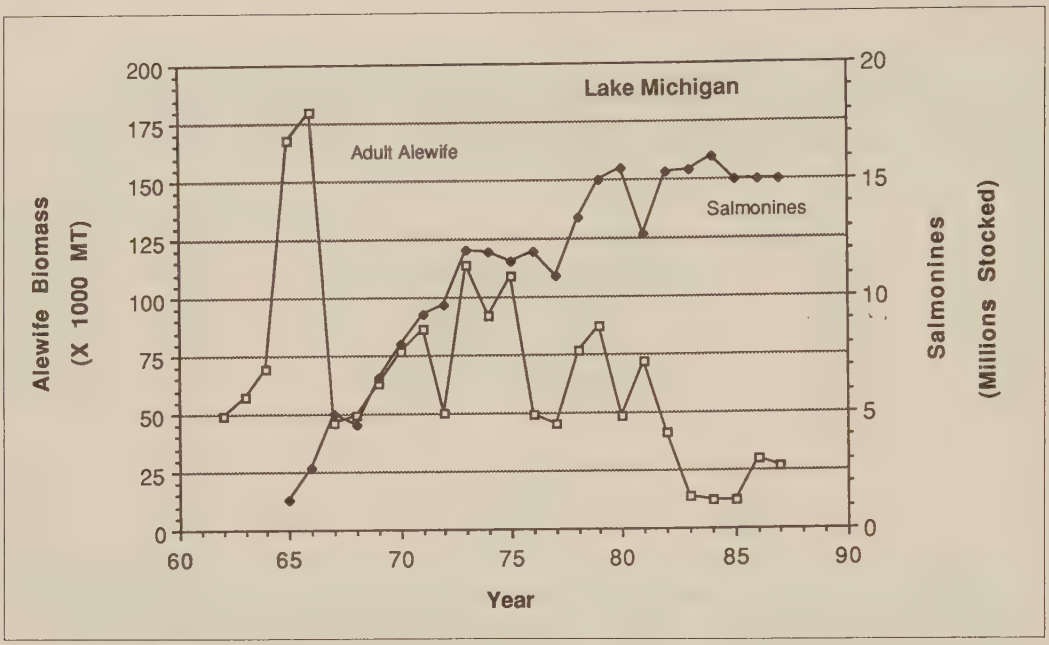

Figure 1

Estimated biomass of adult alewife and numbers of juvenile salmonines stocked in Lake Michigan for the period of 1962–87. Alewife data are extrapolations from bottom trawl surveys reported by Wells et al. (1987). Estimates of alewife abundance during the period of 1962–72 are based on a composite of sources presented by Hatch et al. (1981) and Wells (1985).

fishery estimated to yield economic benefits approaching U.S. $3–4 billion per year in the Great Lakes region (Talhelm 1987).

Estimating Carrying Capacity

In the early stages, salmonid stocking was limited only by hatchery production capacity which grew rapidly in response to public demand. Fishing was excellent, alewife was the predominant prey and the frequency and intensity of alewife die-offs decreased. As stocking rates increased in Lake Michigan, it became evident that introduced salmonids might act as keystone predators and begin to restructure the forage community through increased predation on alewife. Given the tremendous effect of the alewife invasion on the other components of Lake Michigan's food web, a complex series of events might transpire if alewife were to decline (Kitchell and Crowder 1986).

While traditional fisheries research is based on waiting for data, the importance of the Lake Michigan fishery called for alternative approaches that might help anticipate effects and offer some guidance to research and management activities. We (Stewart et al. 1981) attempted to evaluate the nature and magnitude of potential predator-induced changes using bioenergetics modeling to estimate total predatory demand on alewife in Lake Michigan. Estimates of alewife biomass were derived from the trawl surveys reported by Hatch et al. (1981), Wells (1985) and Wells et al. (1987). Based on extrapolations of the intended stocking policy and estimates of alewife production capacity, we predicted a decline in alewife stocks (Stewart et al. 1981). Shortly thereafter, alewife stocks declined precipitously and have remained at a fraction of their abundance during the period 1966–80 (Fig. 1).

Based on ecological reasoning, other predictions regarding responses to alewife decline included reduced growth rates and increased diet breadth of salmonids, increased abundance of large zooplankton owing to the reduction in zooplanktivory, and a resurgence of the native planktivores that were suppressed by competition-predation interactions during the alewife population explosion of the 1960's (Stewart et al. 1981). These forecasts have been tested and documented (Kitchell and Crowder 1986; Scavia et al. 1986) although there are credible alternative explanations that may have contributed to the decline of alewife (Eck and Wells 1987).

It appears that predation pressure by piscivores stocked in Lake Michigan may be approaching the capacity of the system. This view is evident in the current stocking policies of both the Wisconsin and Michigan Department of Natural Resources. Their procedures derive, in part, from an energetics modeling approach which is used to determine stocking rates and species mixes that will keep total

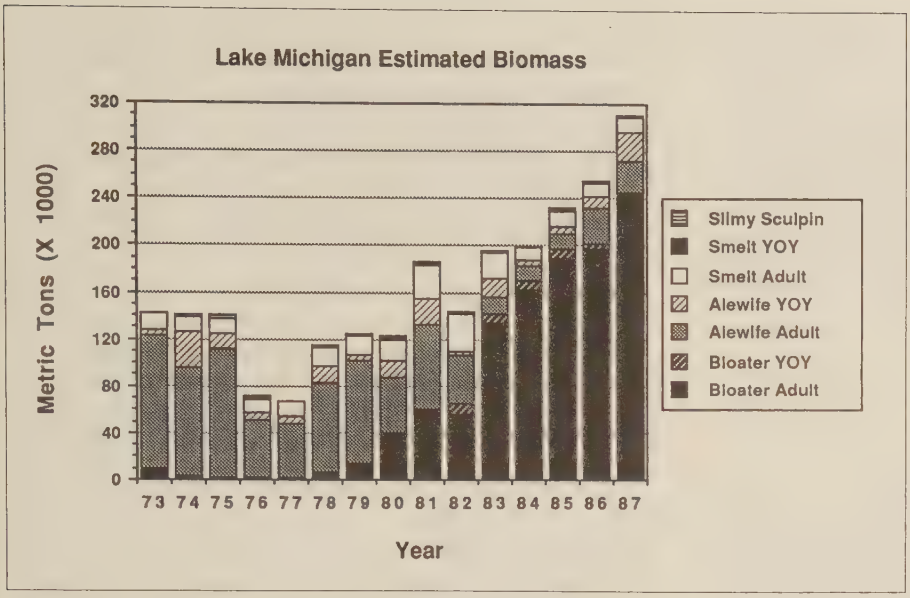

Figure 2
Estimated biomass of forage species in Lake Michigan. Data from Wells et al. (1987). Yellow perch are not included in these data but have generally increased in parallel with populations of the bloater chub (Eck and Wells 1987).

predation pressure at levels evident in 1982 (Krueger and Dehring 1986). This equates to a stocking rate of about 15 million salmon and trout "equivalents" per year.

As alewives have declined, populations of many planktivores and benthivores in Lake Michigan have increased, most notably yellow perch (*Perca flavescens*), bloater chub or deepwater cisco (*Coregonus hoyi*), rainbow smelt (*Osmerus mordax*), and deepwater sculpin (*Myoxocephalus thompsoni*) (Wells et al. 1987). Both sport and commercial fisheries, which declined during the period of alewife dominance, are again developing for perch, chub, and smelt. Their relative abundance is notably different between inshore and offshore areas. Offshore, alewives have been replaced primarily by bloater chub and rainbow smelt (Fig. 1), whereas yellow perch now predominate inshore (Jude and Tesar 1985; Wells et al. 1987). Quantitative estimates of abundance for these resurgent species are not available, but it appears that total fish biomass has increased (Fig. 2). Difficulties in estimating abundances are due, in part, to differences in the distribution of these species and the selectivity of bottom trawls used for assessment (Eck and Wells 1987; Wells et al. 1987). Alewives are more pelagic whereas bloater chubs and yellow perch are primarily demersal and thus may be more available to bottom trawls. As the alewife has declined, resource partitioning among the native species (Crowder 1980) may have allowed development of a greater total fish biomass than that observed in the early 1960's when alewife comprised 90% of the total (Wells et al. 1987).

Changes in the species composition of the planktivore and benthivore communities and their effects as predators in the Lake Michigan food web have had profound effects on the plankton community. Using paleoecological evidence, Kitchell and Carpenter (1987) reconstructed the historical food web which changed dramatically as alewife expanded in the 1960's. More recently, as alewife have declined, yellow perch populations have increased the level of zooplanktivory resulting in a 10-fold decrease of inshore zooplankton biomass since 1978. However, there was no change in species composition, as a small cladoceran, *Bosmina longirostris*, continues to dominate (Evans 1986). Offshore, the switch to bloater chubs, with their hypolimnetic distribution and benthic food habits, has directed predation pressure away from the epilimnetic zooplankton. This produced an abrupt shift in the offshore zooplankton community. Between 1982 and 1983, dominance switched from calanoid copepods (*Diaptomus* spp.) to large *Daphnia* spp., although total zooplankton biomass remained similar (Evans and Jude 1986; Scavia et al. 1986). Daphnids are much less selective and more efficient grazers than are calanoids thus, total phytoplankton grazing rates have increased dramatically since 1982 (Scavia et al. 1986). A shift occurred in species composition of offshore phytoplankton parallel with that seen in the zooplankton. Prior to 1983, summer epilimnetic phytoplankton was dominated by filamentous and colonial blue-green and green algae. Since 1983, dominance has switched to small cryptophytes (Fahnenstiel and Scavia 1987a). Total phytoplankton carbon concentration has remained stable over this period, although subthermocline phytoplankton communities have increased (Fahnenstiel and Scavia 1987b). The result of these changes in the offshore plankton community is increased water clarity. Maximum summer secchi disk readings have increased from 4 to 5 meters in the mid-1960's (Lesht and Rockwell 1985) to 15 meters or more in recent years (Scavia et al. 1986).

Thus, the story of Lake Michigan is one of dramatic change in community composition and species abundance

at different trophic levels resulting from strong interactions among food web linkages. These changes were initiated, both in the early part of the century and recently, by dramatic changes in the abundance of top predators and the predation pressure they exerted down through the food web. While the decline in predators in the early part of the century was a relatively inevitable event, the recent increase in predator abundance has been directly controlled by man. Changes in water quality over time have resulted from a synergism of nutrient abatement and food web interactions.

Management of Dynamic Systems

Within the dynamic ecological framework described above, the Great Lakes Fishery Commission provides an institutional opportunity for development of a management policy that can include consideration of the species interactions represented in a complex food web. The Great Lakes Fishery Commission coordinates management and research activities involving the agencies of 11 Great Lakes states, the Province of Ontario, and the federal governments of both the U.S. and Canada. In addition, the Sea Grant Programs in each of several states offer the benefit of university-based research programs focussed on Great Lakes resources. One example of policy that derived from the collaboration of research and management interests is represented by the planning document produced by the Wisconsin Department of Natural Resources (1986). It defines a series of objectives for managing Lake Michigan fisheries that include

- reestablishing a self-sustaining lake trout population through continued stocking and reductions in sport and commercial fishing mortality;
- limiting commercial harvests of bloater chub to 4 million pounds and of yellow perch to 200,000 pounds;
- increasing sport harvests to 400,000 yellow perch and 650,000 trout and salmon per year;
- placing more emphasis on rainbow trout enhancement and a trophy fishery (fish > 22 kg) for sterile chinook salmon.

The states of Michigan, Illinois, and Indiana have similar policies with some amendments for local interests.

Meeting these objectives will require changes in stocking policies to produce changes in the relative abundances of salmonid predators and the distribution of predation pressure over space and time. Bioenergetics models have been developed to aid the planning process as represented in recent work on the role of sterile salmon. Sterile chinook salmon, which were first stocked in 1986, will live up to 10 years, attain trophy weights of 45 kg by 1994, and consume about 1.5 times as much forage as a cohort of normal chinook salmon (Kitchell and Hewett 1987). Similar analyses are underway in the current evaluation of the stocking policy for anadromous rainbow trout (steelhead). The Wisconsin Department of Natural Resources (1986) will seek to enhance shore and stream fishing opportunities through the use of three different seasonal-run strains: a spring run (Ganarasha), summer run (Skamania), and fall run (Chambers Creek) strain. These fish are all expected to be epilimnetic, but differences in summer temperature preferences and distributions are not known. The three strains will be stocked at different ages (12–16 months) and will have different residence times in the lake (from 2 to 3 years). As demonstrated by Stewart et al. (1981), differences in thermal preferences and life histories have very large effects on total predation patterns.

Changes will also occur in mortality patterns of Lake Michigan salmonines. Lake trout have been specifically targeted for reductions in harvest mortality through a decrease in the sport bag limit, a shortened season, a closed area in the western part of the lake, and numerous changes in the commercial fishing regulations. These reductions in harvest will very probably result in larger lake trout populations and in compensatory increases in sport harvest of other salmonines. Some salmonines, particularly coho and chinook salmon, have already experienced a shift in seasonal patterns of mortality. In the 1970's, sport harvest of salmonines occurred mostly in nearshore areas during the fall spawning run (Stewart et al. 1981). In contrast, Kitchell and Hewett (1987) recalculated mortality schedules based on reports by Bleser and Hansen (1985). They found that harvest was occurring earlier in the season and in deeper, offshore areas. In effect, fish are being harvested at a younger age, thus their total predatory impact is lessened by an initial estimate of 30–40% per year. This change contributes to the recent increase in alewife observed since 1985 (Fig. 1).

Successful natural reproduction by salmonines could act as a destabilizing influence on the fish community. Reproduction by rainbow trout, plus coho, chinook, and pink salmon, has occurred in some tributary streams of northern Lake Michigan. This coupled with possible lake trout reproduction represents a new, variable input to the total predator biomass and thus reduces our ability to control predator biomass (and predation pressure) through manipulating stocking rates and fishing regulations.

Changes in the relative abundances, life histories, and mortality patterns of lake trout and other salmonines will impose new temporal and spatial patterns of predation on the forage base. Shifts in predation pressure away from alewives and toward alternate prey species (e.g., smelt, chubs, and perch) could impinge upon commercial and sport harvest objectives for those species. Obviously, the reverse is also true if commercial fisheries compete with salmonine predators for prey resources.

Prospects for the Future

The resiliency of alewife, the primary prey, in these systems is unknown. The dynamics and potential of this exotic species are a key to effective management for the future. Like many clupeid fishes, its population dynamics are volatile and defy prediction based on traditional fisheries models. There is no doubt that hard winters can cause higher alewife mortalities (Eck and Wells 1987). However, the magnitude of that factor remains confounded by density-dependent intraspecific competition and the depensatory effect of a highly efficient predators (salmonines) whose populations are dictated more by management policy than the ecological principles of typical predator-prey systems. Alewives were 90% or more of salmon and trout diets in the 1960's and still constituted a major portion (70–80%) of the diet in the mid 80's after their populations declined (Hagar 1984) indicating an apparent preference for alewives over other forage species. Given this preference along with current levels of salmonid stocking and intensified competitive interactions among forage species (Fig. 2), it seems unlikely that alewives will return to former levels of abundance. They may exist at low levels in the system or may exhibit recurrent outbreaks associated with strong year classes.

Salmonid stocking also resulted in responses higher in the trophic system. Sizes of spawning-run adult sea lampreys approximately doubled over the two decades of continuous growth in the salmonid stocking program. While the sea lamprey population is controlled by the lampricide treatment program and remains low, the parasitic or predatory effect of an individual sea lamprey has increased nonlinearly and by about sixfold (Kitchell 1990).

Thus, it appears that Lake Michigan is entering a new phase of management. The goal is no longer simple expansion of the predator complex to control alewife populations and produce more fish for sport anglers. Managers must now regulate the top predator levels, considering not only the total predation pressure within the system, but how that pressure is distributed over different predators and forage species. Manipulating top predator communities provides an effective means of managing not only those species of direct interest to fisheries, but also of effecting changes in lower trophic levels through directed predator-prey interactions that can ameliorate competition-predation effects, facilitate recovery of native species, and enhance water clarity (Kitchell et al. 1988).

Among the tools available to management and research scientists, bioenergetics modeling (Hewett and Johnson 1987) can be used to quantitatively address questions of predation rates and production by an individual predatory species or a suite of predators within a single trophic level (e.g., Kitchell et al. 1977; Kitchell and Breck 1980; Stewart et al. 1981; Stewart et al. 1983; Rice and Cochran 1984; Bartell et al. 1986; Olson and Boggs 1986; Stewart and Binkowski 1986; Kitchell and Hewett 1987; Kitchell 1990). It differs from other modeling techniques in that it does not depend upon the *a posteriori* assessments of traditional fisheries models, has a firm quantitative basis, and can be used to test and strengthen the assumptions of simulation modeling. We must now expand this approach to consider how changes in management policy are expressed throughout the food web (i.e., in piscivores, planktivores, benthivores, and plankton). Development of an ecological rationale for managing Lake Michigan fisheries will require improved understanding of seasonal and long-term production dynamics of the major forage fishes as well as quantification of fluxes between trophic levels. As indicated above, this approach can also yield benefits in water quality. The larger goal for long-term management is to develop the research perspective, analytical tools, and practical guidelines that will allow managers to put strategic planning into tactical practice.

Bioenergetics modeling can provide valuable insight into predator-prey interactions under different community structures. But, which of the possible structures is most likely to represent a stable configuration within the productive capacity of Lake Michigan? We can begin to address this question by examining indicators of past community structure in the lake. Zooplankton community dynamics are widely recognized as highly responsive to predator effects and have been successfully used as indirect and integrative sources of insights for the entire ecosystem (Kitchell and Kitchell 1980; Kerfoot 1981; Mills et al. 1987; Carpenter and Kitchell 1988). Our recent paleoecological work on Lake Michigan sediment cores showed that these sediments contain zooplankton remains which provide archival evidence of past predator-prey states and dynamics (Kitchell and Carpenter 1987). The mean length of the mucron (the posterior spine) on *Bosmina longirostris* carapaces decreased dramatically in the sediment core at a depth representing sediments deposited around 1960, the time of rapid increase in alewife abundance. Prior to 1960, zooplanktivory in Lake Michigan was relatively low and abundance of predatory copepods was high. The mucron serves as a defense against predation by copepods, thus during this period, long spined morphs dominated. The increase in alewife abundance after 1960 reduced abundance of predatory copepods through size-selective predation and the short spined morph of *Bosmina* became abundant. The most recent analysis of open-water plankton samples from Lake Michigan indicates that as abundance of alewife has declined and predatory copepods have increased, *Bosmina* mucron lengths have increased and are currently about twice as long compared to the 1950's (Kitchell, unpubl. data). This indicates that current levels of zooplanktivory in Lake Michigan are likely lower than those under any ancestral condition. Thus, *Bosmina* morphology over time, as reconstructed from sediments and open-water samples, documents the advent, dominance, and recent decline of

the impact of alewife on the Lake Michigan zooplankton community. It also appears that *Bosmina* morphology is an indicator of alternate steady-state behavior of the predator-prey system. The morphological response exhibited in Lake Michigan was neither gradational nor linearly related to alewife effects and suggests a combination of switching and depensatory mechanisms often observed as systems change state (Holling 1978; Walters et al. 1980).

Events similar to those in Lake Michigan have also occurred, to varying degrees, in each of the Laurentian Great Lakes. For example, there have been sharp declines in the alewife population of Lake Huron (Argyle 1984), and in Lake Superior, smelt declined while lake herring (*Coregonus artedii*) increased (Selgeby 1985). A decline in body condition of lake trout in western Lake Superior may have been due in part to the smelt decline. In both Lake Superior and Lake Huron, declines in the forage fishes occurred as salmonid stocking rates reached high levels (Selgeby 1985). In Lake Ontario, salmonid stocking rates increased rapidly in the late 1970's and yet another highly successful sport fishery developed (Hartig et al. 1991). Density-dependent effects appeared to be the primary regulator of alewife dynamics (O'Gorman et al. 1987). However, concern for the forage base (primarily alewife) and the lessons learned elsewhere have caused the fisheries agencies involved to adopt a "hold-the-line" policy since 1985 while large cohorts of recently stocked salmon come to maturity and have their maximum predatory impact (Hartig et al. 1991).

Each of the Great Lakes have different levels and stages of predator-prey interactions, but all within the same basic ecological framework. Comparisons between systems can help determine Lake Michigan's status relative to the other lakes. Principles derived from Lake Michigan can then be modified for application to other systems. While the lessons from Lake Michigan's history may be readily applied among the Great Lakes, their application to different systems will require an astute combination of experience, insight, logic, and modeling.

The dimensions, complexities, and compensatory potential of large marine systems make it difficult to imagine that stocking policies might have a readily apparent impact on marine food webs. However, we should not ignore the many examples of predator effects demonstrated in the scores of over-exploited marine fish stocks and the compensatory responses of communities selectively altered by fishing. In my opinion, effects of selective and intensive fishing on marine food webs must have occurred but remain poorly documented. It follows that extensive stock enhancements, such as those due to the salmon enhancement programs, should receive the attention of the research community.

Perhaps a very important lesson to be learned from the history of the Great Lakes can be conveyed by anecdote. A recent discussion with one of the most experienced of Great Lakes fisheries biologists (LaRue Wells, now retired from U.S. Fish and Wildlife Service) turned to the question of which of the many changes observed in Lake Michigan were fully anticipated by researchers and managers? His answer can be paraphrased as: "Only one—that was the effect of sea lamprey on lake trout. We knew that was coming because we'd seen it in the other lakes." Since then we have developed a greater understanding of basic ecological principles as they apply to large pelagic systems and can use those as a basis for testable forecasts (Kitchell and Crowder 1986; Carpenter and Kitchell 1988). Some of those forecasts are wrong. That, however, is the essence of science and the essential requisite if we are to learn how ecosystems work and how the lessons from one place may be generalized in applications to others.

The Great Lakes now offer us the opportunity to learn from history as we consider the cascade of effects that may descend through food webs enhanced by the continuous addition of large predators. The salmon enhancement programs currently underway along the North Pacific rim offer the chance to observe ecological analogues in a large pelagic marine environment. Logically, we should attend to that prospect in planning for research and management programs.

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Farming Techniques for Bay Scallop, *Pecten (Notovola) albicans*, in the Western Regions of the Japan Sea

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ABSTRACT

The bay scallop, *Pecten (Notovola) albicans*, is an important resource in the coastal waters of Japan. Scientists with the Marine Ranching Research Program are studying bay scallop ecology and developing new methods to improve its survival, growth, and harvest. This paper discusses the natural mechanisms controlling these outcomes and also introduces a new bottom-type spat collector that greatly improves the collection of settling larvae. It is suggested that the number of hanging cultured shells be increased in order to increase bay scallop seed resources for their higher fecundity.

Introduction

The bay scallop, *Pecten (Notovola) albicans*, is distributed from the southern coast of the Aomori Prefecture to the Tsushima coast on the Japan Sea. The annual catch of this species fluctuates remarkably. In the Marine Ranching Research Program, we have developed new techniques useful in bay scallop farming.

The purpose of this research is to improve bay scallop production by increasing our knowledge of 1) the natural mechanism controlling the concentration, deposition, and survival of swimming bay scallop larvae in the sea and 2) the relationship between spat deposit-site conditions and their corresponding survival rates.

Materials and Methods

Research was conducted in the coastal areas of Tottori and Shimane Prefectures from 1980 to 1988. The project team consisted of four laboratories: The Japan Sea Regional Fisheries Research Laboratory, Nansei Regional Fisheries Research Laboratory, and the Tottori and Shimane Prefectural Fisheries Experimental Stations.

A total of 65 research stations were established for collecting samples.

Swimming Larvae Collection

Each laboratory collected swimming larva using a NXX 13 nylon cloth NORPAC net (diameter 45 cm, side length 180 cm), which was hauled vertically from 30 or 50 m to the surface. Nets were retrieved at the rate of 10 cm/sec and weighted with 5 kg of ballast.

All of the 540 samples were collected over an 8 year period from December through May. Samples were treated with 5% formalin and examined microscopically in the laboratory.

Establishment of Sea Currents

Generally, the number one branch of the Tsushima Warm Current in the Japan Sea flows by the northern coast of Honshu. The surface water temperatures, which range from 11.00 to 15.18°C from December to May, are generally stable to a depth of 50 m during the collection period.

The NOAA satellite images (AVHRR, Advanced Very-High Resolution Radiometer) of surface water temperatures in the range of 6.85 to 15.69°C were almost equal to the layer in which the swimming bay scallop larvae were found.

The upwelling of cold water masses and sea bottom topography combine with the currents of the first branch of the Tsushima Warm Current to make several eddy zones. In the upwelling cold water in the Japan Sea at Hashizu in Tottori Prefecture, phosphate (PO_4) is stable at about 0.40–0.60 ppm (Tanaka et al. 1981).

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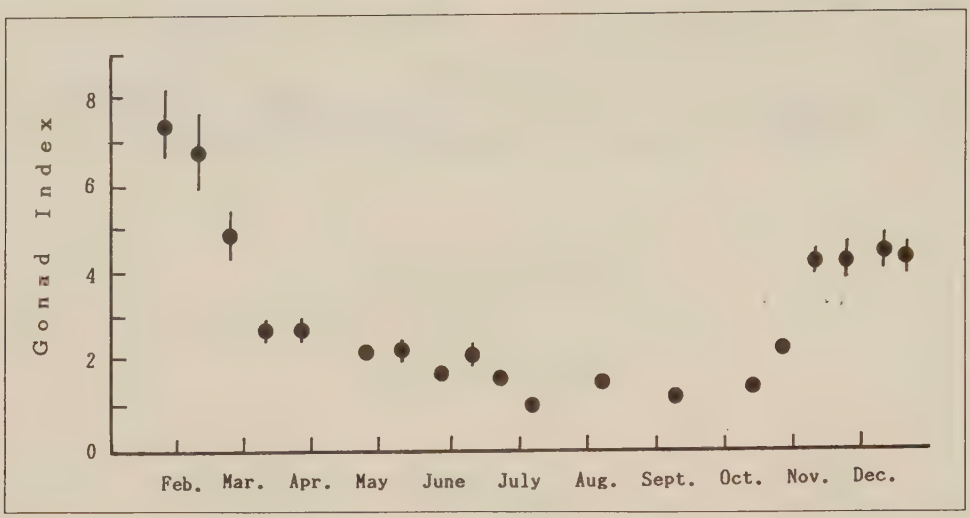

Figure 1
Seasonal change of gonad index of bay scallop (Moriwaki et al. 1981).

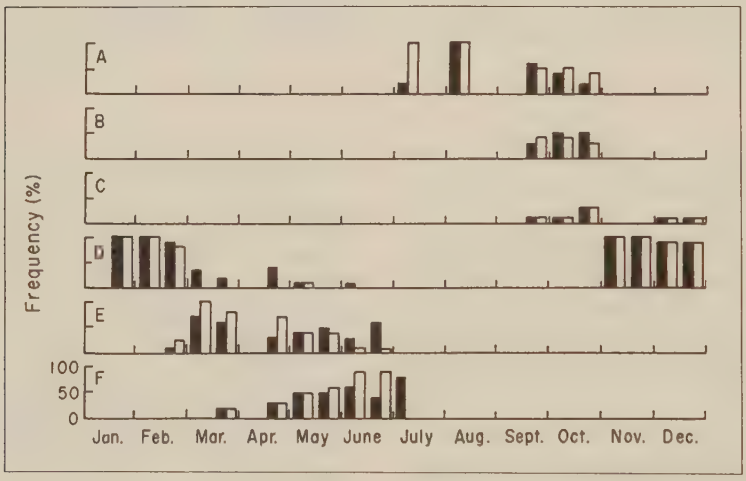

Figure 2
Seasonal change of gonad development of bay scallop (Moriwaki et al. 1981). A: premature stage; B: follicular stage; C: growth stage; D: mature stage; E: spawning stage; F: spawned stage; ▮: ovary, and □: testis.

In the warm current, it is only 0.10–0.20 ppm. Ammonia levels (NH_4) are stable at 1.0–2.0 ppm in the cold water mass.

The difference in phosphate-P concentration dissipates as one moves from the cold water mass.

Gonadal Indices

Natural and cultured bay scallop gonadal indices have been developed using either dredged or purchased samples taken on a monthly basis from the coasts of the Oki Isles, Shimane Peninsula and Tottori Prefecture (Moriwaki et al. 1981; Tanaka et al. 1985, 1986a). Gonadal development was determined using microsectioning methods after which specimens were classifed into six ranks by Yamamoto's method.

Deposited Larvae Collection

The new larval collector was constructed of an onion bag, mesh size 2–4 mm, filtered with a collection material made of hard nylon fibers from a 50–70 mm mesh size fishing net. Each collector was set on the sea bottom using a basket-like crab pot (3 × 4 × 2 m) and a long line approximately

Table 1
Development and growth of bay scallop. (Hotta 1977.)

Development	Diameter size or shell length	Characteristics
Fertilization of egg	78.8 μm	submerged globular form
2 days after	104 μm	D-shaped swimming larva
10 days after	120~160 μm	Umbo stage growth, 4.0-5.2 μm/day
14 days after	190~210 μm	Umbo stage growth, 7.3-8.3 μm/day
20 days after	154~270 μm	Eye spot, velum appears
25 days after	154~270 μm	Deposited by byssus
28 days after	230~250 μm	Marginal shell formed
1 month after	315~356 μm	8.6-16 μm/day growth
2 months after	1.5~3.6 mm	20-140 μm/day growth
3 months after	10 mm	Benthic life, 2.0-3.0 mm/day growth
6 months after	5 cm	Adult shell form, 14 g BW
1 year later	8 cm	Adult shell form, 60 g BW
1.5 years later	10 cm	Adult shell form, 100 g BW
2 years later	11 cm	Adult shell form, 150 g BW
2.5 years later	12 cm	Adult shell form, 200 g BW

Table 2
Relation between bay scallop and other bivalve larvae (individuals/m^3). (Tanaka et al. 1982, 1983.)

Date	Bay scallop larvae		Other bivalve larvae		Appearance of bay scallop (%)
	Range	Average	Range	Average	
March 1980	0~9	1	0~117	29	3.4
April 1980	0~20	4	8~338	68	5.8
April 1981	0~5	1	0~145	45	2.2
May 1981	0~3	1	0~235	72	1.4
December 1981	0	0	10~394	136	0
March 1982	0~13	2	2~218	23	8.7
April 1982	0~122	11	3~1415	157	7.0
May 1982	0~2	0	10~274	68	0

50-80 m in length. The level of the total organic carbon (TOC) of muddy settling substrates was measured using a TOC meter; ignition losses of mud were measured at 550° C for one hour using an electric muffle hearth.

Results and Discussion

Spawning Season and Swimming Larvae Stages

Off the coast of the San-in district, the bay scallop's spawning season is from November to March (Figs. 1, 2; Moriwaki et al. 1981). The swimming-larvae stages follow fertilization by several days, last 3 or 4 weeks, and occur primarily in shallow water less than 50 m in depth (Table 1; Hotta 1977). Larvae accumulate in the Tsushima Warm Current as their growth continues. Bay scallop larvae range from 0.4 to 8.1% of the total bivalve larvae population, averaging 4.2% (Table 2; Tanaka et al. 1982, 1983). The peak of veliger larvae distribution was found in December and February (Fig. 3; Yuki et al. 1985).

The mechanism of spawning larvae accumulation can be explained as follows. The swimming larvae distribute in the middle layer of the first branch of the Tsushima Warm Current (Fig. 4; Kasahara and Itoh 1985) which flows to the east of this experimental district. Along the Honshu coast around the Oki Isles, a sea valley exists from the Tottori city to the eastern coast of the Nishinoshima Oki Isles. Here an upwelling cold water mass was observed which controlled the first branch of the Tsushima Warm Current flowing eastward (Fig. 5). The formation of an eddy was observed in this zone (Fig. 6) wherein the bay scallop swimming larvae concentrated (Fig. 7).

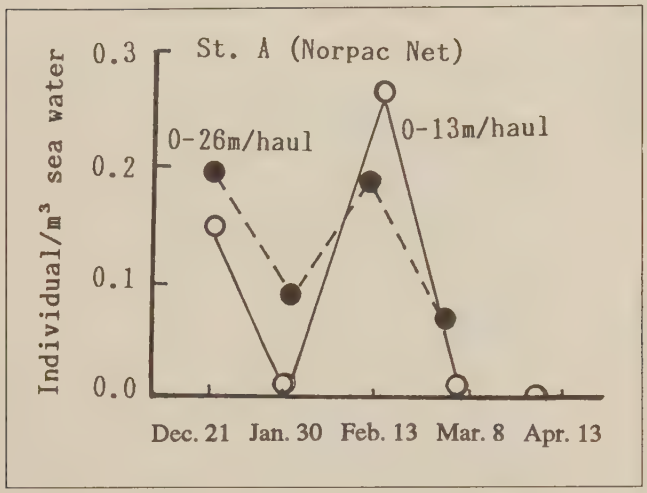

Figure 3
Distribution of swimming larvae for each month at Stn. A (Yuki et al. 1985). ----- = 0–26 m a haul; ——— = 0–13 m a haul.

We also estimated the areas of concentrated swimming larvae (Fig. 8, 9; Tanaka et al. 1986b) using NOAA satellite images of surface water temperatures when the collection of larvae was difficult owing to stormy conditions. Presently, satellite imaging using infrared rays can be displayed with a 3.1-km mesh, enabling us to detect the distribution area of the swimming larvae. The maximum density of the swimming larvae was 122 individuals per cubic meter of sea water. This density is very low compared to the Japanese scallop (*Pacinopecten yessoensis* Jay) in Mitsu Bay. The supply may be affected by the swift currents involved.

Deposition of Bay Scallop Larvae

As the swimming larvae increase to 0.3–0.5 mm in shell length, they settle to the bottom. Before reaching the sea bottom, the larvae attach to suitable materials by attachment of the byssus. If suitable materials are not present, they settle down on the sandy or muddy bottom characteristic of the slow current areas of eddy zones. Larvae deposited in the muddy conditions usually cannot survive (Tables 3, 4; Fig. 10). We have forecasted that larvae of 23.25 ± 1.80 to 3.50 ± 0.60 mm in shell length might be unable to survive in the mud more than 2 or 3 weeks. It is not surprising therefore that the 0.3 to 0.5 mm shell larvae deposited on the mud also could not survive. It is suggested that the bottom materials in the deposition zone are an important survival factor, and environments high in organic matter and silt content are unsuitable for larval development.

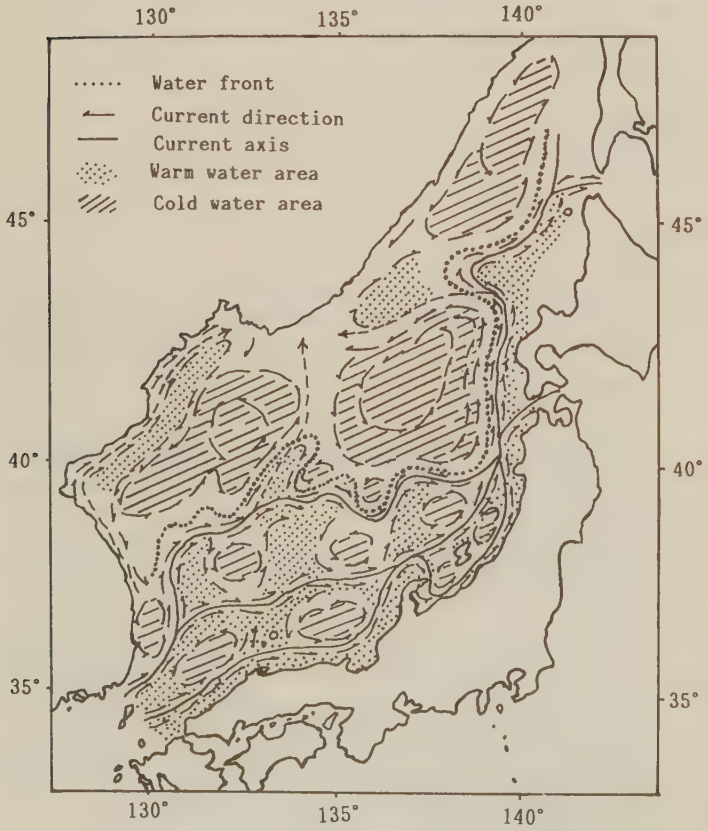

Figure 4
Sea current of the Sea of Japan by Nagamuma's current pattern. (Kasahara and Itoh 1985.)

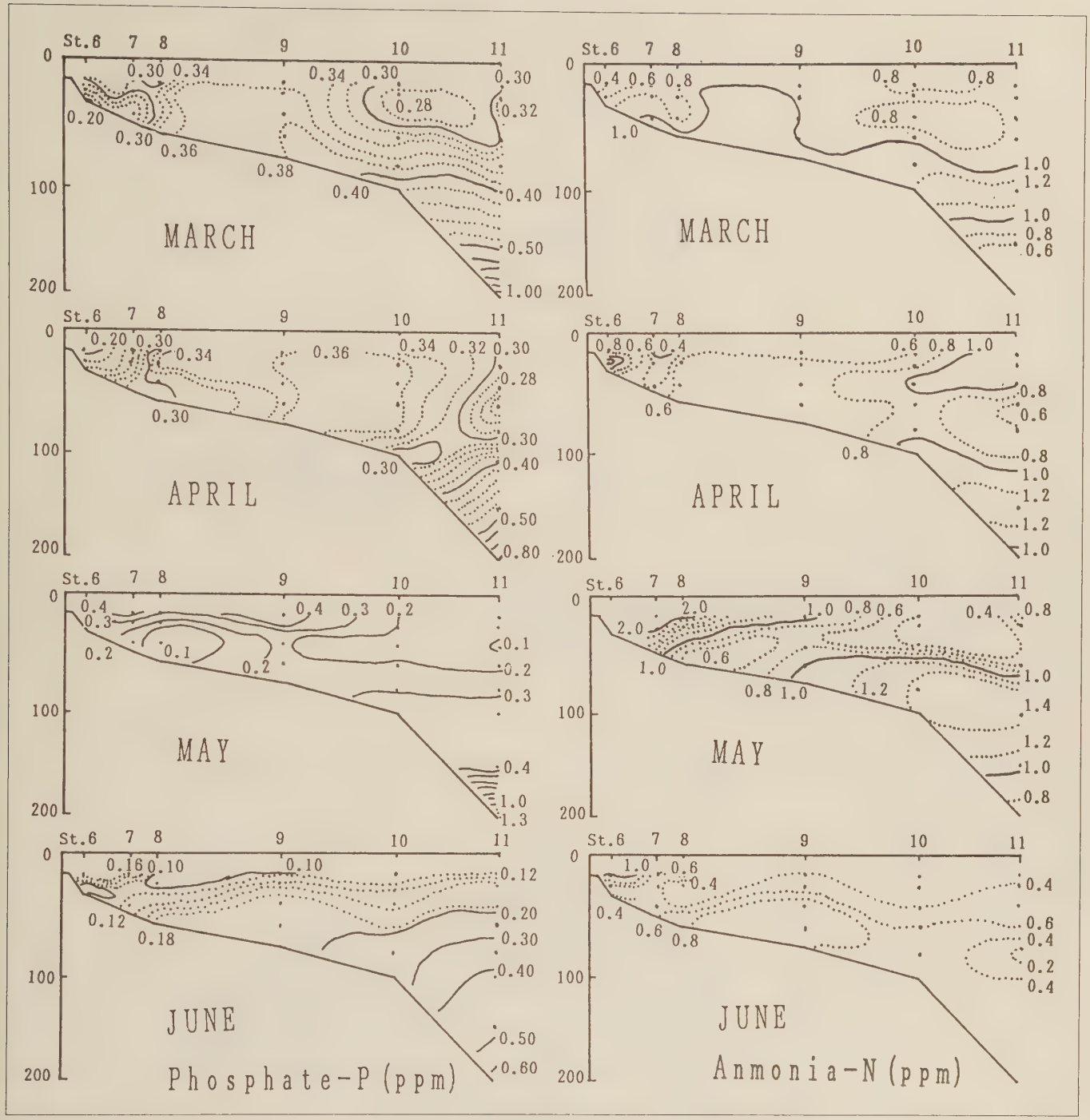

Figure 5
Vertical distribution of PO_4-P (left) and NH_4-N (right) offshore of Hashizu, Tottori Prefecture in 1980 (Tanaka et al. 1981).

Natural Mechanics of Shellfish Survival

The period of swimming-larvae blooming is about the same as plankton blooming. A shortage of food materials, however, does not seem to be the main reason for bay scallop mortality. Significant factors in shellfish survival may include: 1) number of spawned eggs and the fluctuation of spawning term; 2) changes in eddy zone location dependent on the strength of the first branch of the Tsushima Warm Current and of the upwelling of the cold water mass in the Japan Sea by the coast; 3) volume of organic and muddy material in the sea bed deposition

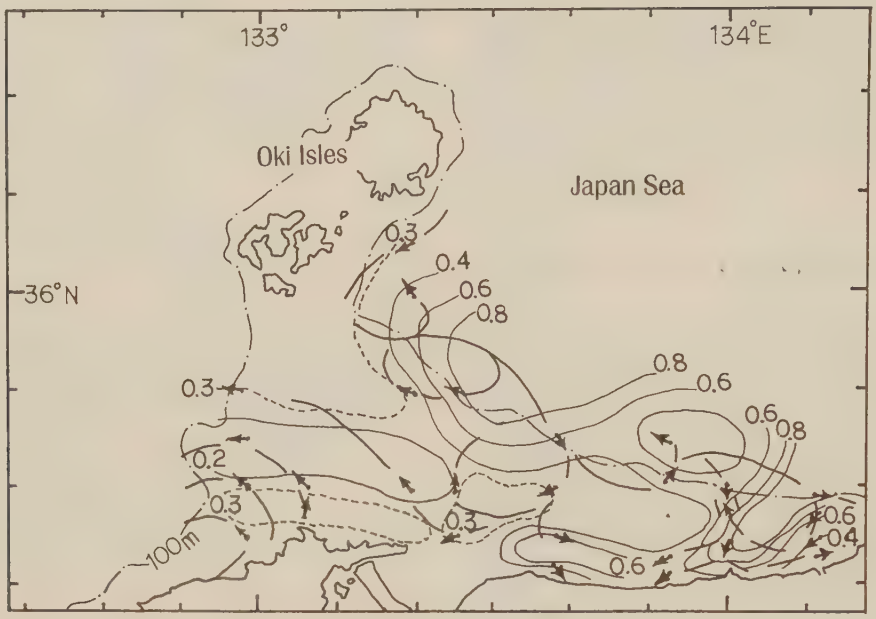

Figure 6

Horizontal distribution of Phosphate-P at the depth of 10 m; arrows indicate current direction as determined by current meter SD-2; phosphate concentration isobars shown in parts per million; — · — indicated 100 m isobath (Tanaka et al. 1981).

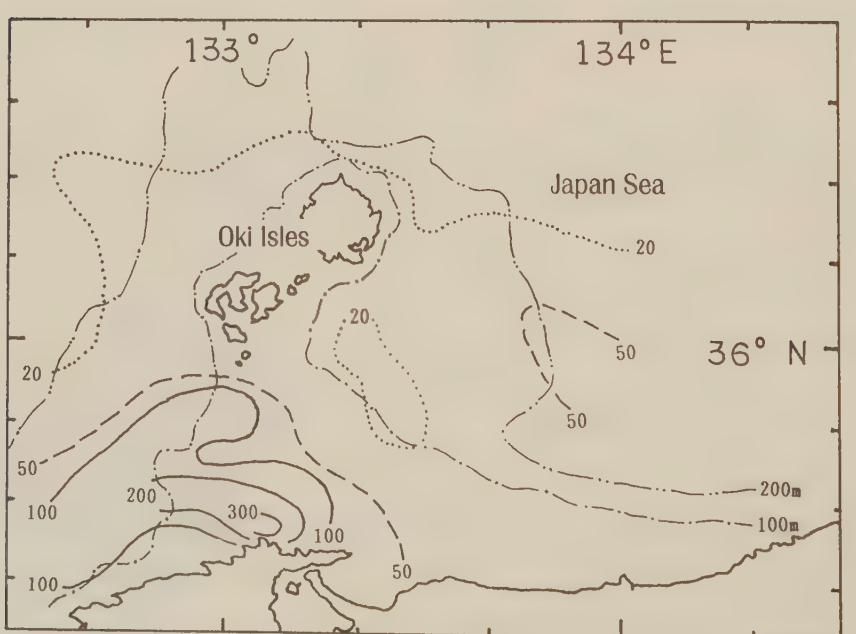

Figure 7

Distribution of bivalve swimming larvae off the eatern coast of Shimane Prefecture around the Oki Islands in the Japan Sea; — · · — indicates 200 m isobath; — · — indicates 100 m isobath; · · · · · indicates 20 larvae/m^3 isobar; ----- indicates 50 larvae/m^3 isobar; —— indicates isobars for larval concentrations >100 larvae/m^3 as shown in figure.

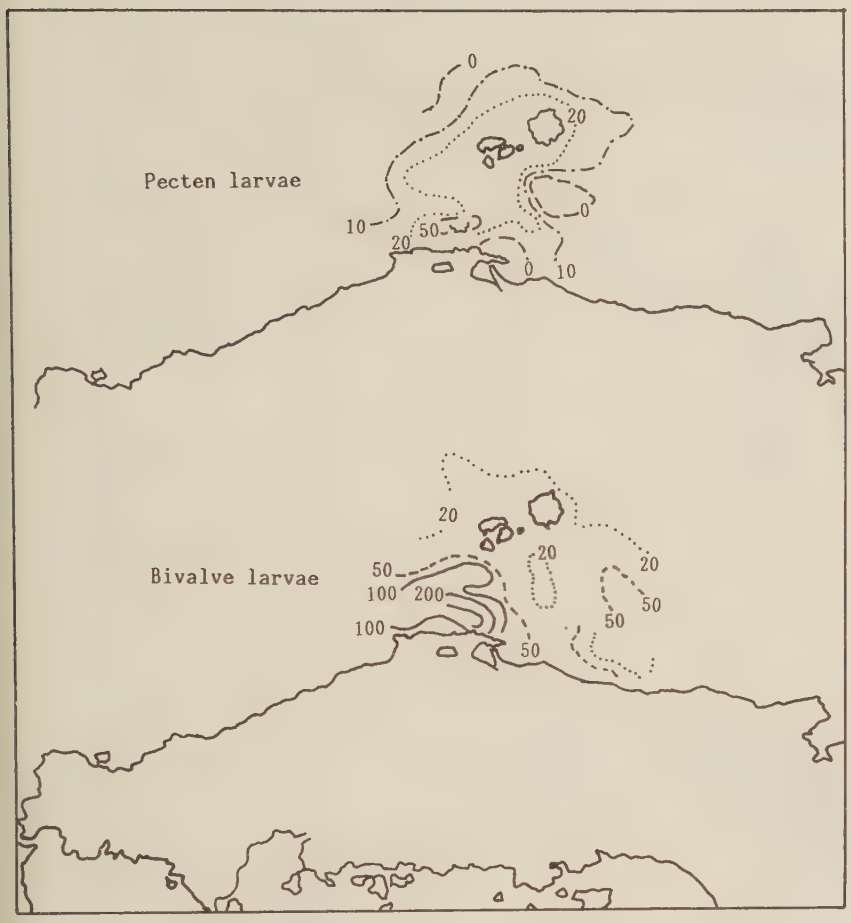

Figure 8
Distribution of bay scallop and bivalve swimming larvae off the eastern coast of Shimane Prefecture around the Oki Islands in the Japan Sea; ····· indicates 20 larvae/m^3 isobar; ----- indicates 50 larvae/m^3 isobar; —— indicates isobars for larval concentrations >100 larvae/m^3 as shown in figure.

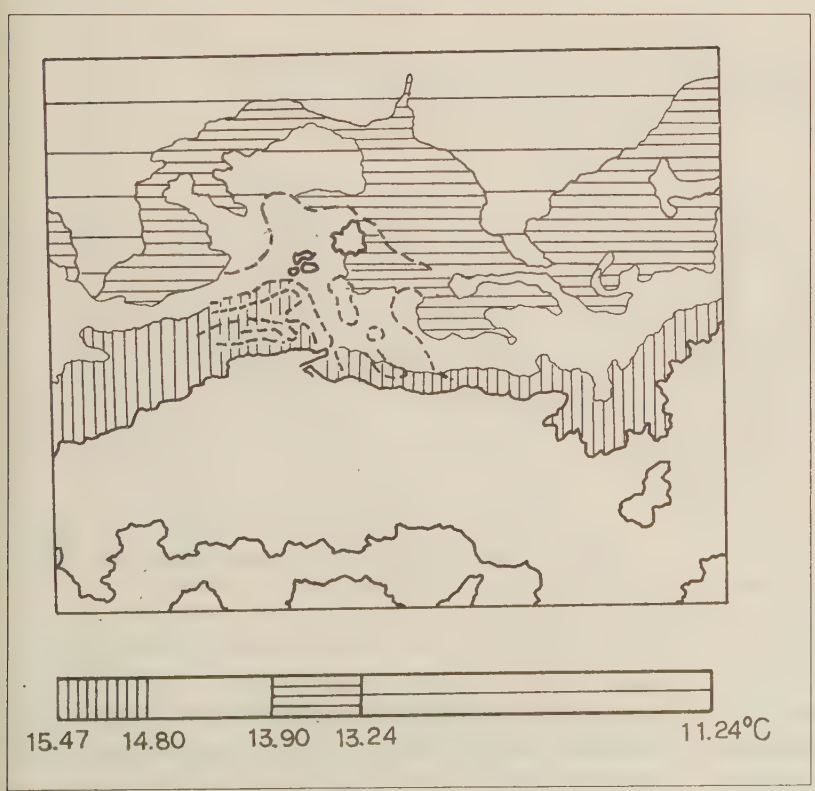

Figure 9
Distribution of surface water temperature by NOAA 6 Polar Orbiting Satellite (Satellite Data Serv. Div. of NESDIS [Natl. Environ. Sat., Data and Info. Service] 5627 Allentown Rd., Camp Springs, MD 20746) in 0.3°C intervals; Dashed lines show the approximate location of isodensity lines seen in Figure 7 (survey conducted on 27 May 1980, 1840 to 1852 hours by NOAA 6).

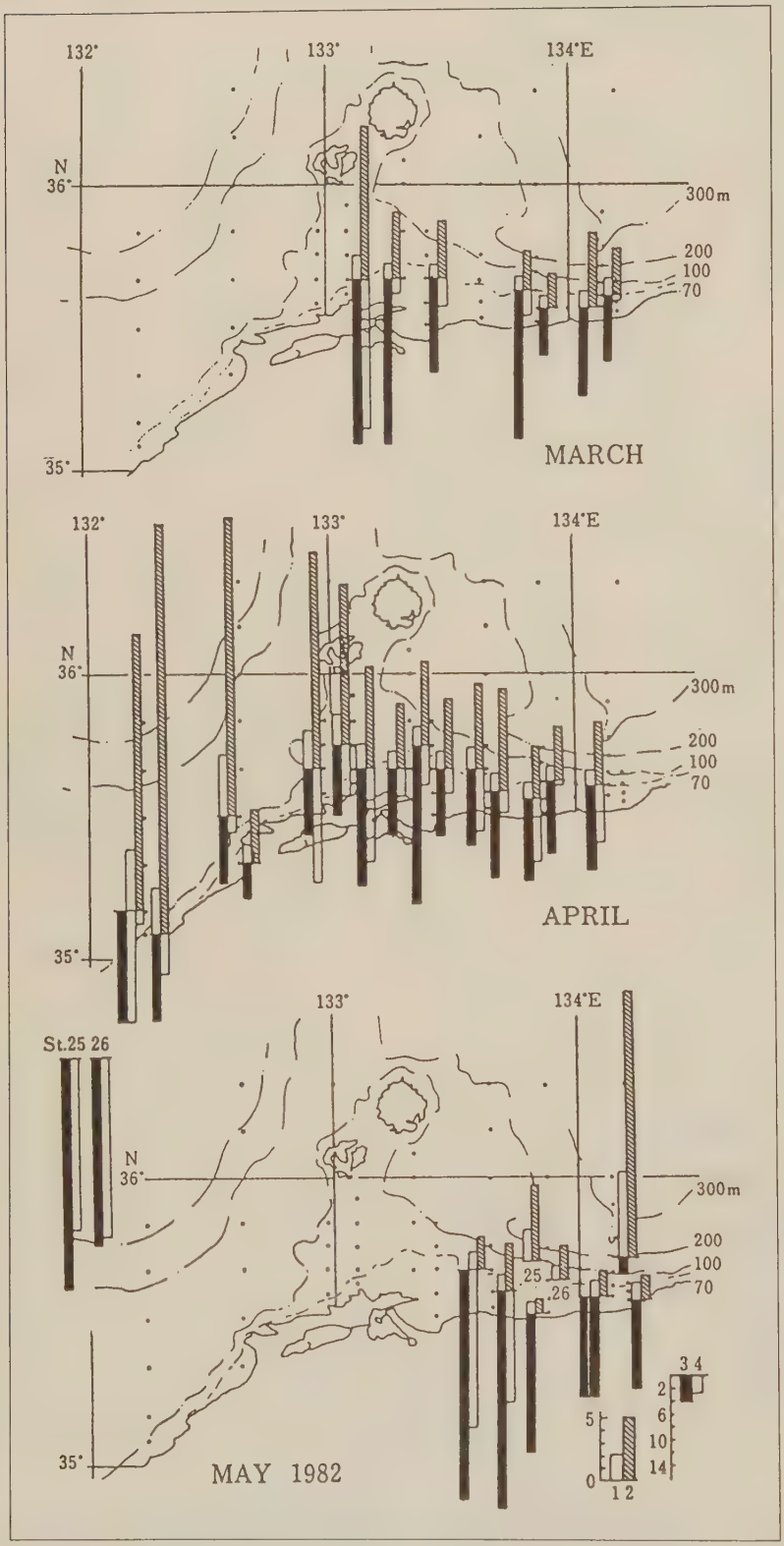

Figure 10

Number of dead demersal bay scallop shells under various conditions off the eastern coast of Shimane Prefecture around the Oki Islands for March through May 1982. The zero point of each bar graph indicates the location of the sampling station location. For each set of bars, top left bar indicates ignition loss of mud (%); top right bar indicates total organic carbon (TOC mg/g dry wt.); lower left bar indicates the total number of dead bay scallops; and lower right bar indicates bay-scallop numbers per kg of mud. Scales for each are shown in the lower right hand corner of the figure; for May 1982, bay-scallop numbers are set off to the left owing to lack of space in the figure.

zone of swimming larvae; and 4) concentration of predators. In other words, the bay scallop quantity is influenced by the survival rate during the early stages of life. Table 5 shows that both large quantities and high densities of bay scallop occur off the San-in coast.

Table 3

Bottom environment characteristics of areas of high juvenile bay scallop mortality in the year 1982. Inds.: individuals; SL: shell length; IL: ignition loss; TOC: total organic carbon.

Date	Sea depth (m)	Dead bay scallop shell: Inds./mud (kg)	Dead bay scallop shell: SL (mm)	Bottom environment: IL (%)	Bottom environment: TOC (mg/g)
March	77	20	2.6	2.4	14.9
April	74	22	2.4	3.7	21.1
April	79	18	2.5	2.5	9.8
April	72	12	2.3	2.1	7.9
April	38	11	4.2	1.5	6.1
April	50	10	2.8	3.0	15.7
April	86	25	2.6	4.6	30.0
Average	68.0	18.1		2.82	15.1

Table 4

Bottom environment characteristics of areas of low juvenile bay scallop mortality in the year 1982. Inds.: individuals; SL: shell length; IL: ignition loss; TOC: total organic carbon.

Date	Sea depth (m)	Dead bay scallop shell: Inds./mud (kg)	Dead bay scallop shell: SL (mm)	Bottom environment: IL (%)	Bottom environment: TOC (mg/g)
March	46	0	—	1.1	3.4
March	40	0	—	1.7	7.4
March	69	1	3.5	1.6	5.5
March	59	3	4.4	1.5	6.6
April	59	1	2.8	1.4	5.1
April	37	0	—	1.9	5.1
April	60	3	4.7	1.7	6.3
Average	52.9	1.1		1.56	5.60

Table 5

The maximum outbreak of bay scallop density on the San-in coast. t: metric tons.

District	Year	Catch (1000 t)	Individuals (10^6)	Area (km^2)	Density (t/km^2)	Density ($Ind./m^2$)
Tottori	1924–25	28	350	453	61.8	0.77
Shimane	1965	18	225	988	18.2	0.23
Ishikawa	1966–67	39	487	1108	35.2	0.44
Total		85	1062	2549	33.52	0.42

Table 6

Comparison of bay scallop spat collection between bottom and hanging method in 1982. Avg. SL: Average shell length (Matsuyama and Takeuchi 1983).

Collector setting date	Recovery date	Bottom collector (A): Deposits (ind.)	Bottom collector (A): Avg. SL (mm)	Hanging collector (B): Deposits (ind.)	Hanging collector (B): Avg. SL (mm)	Ratio (A/B)
February	March	24,932	1.0	185	1.0	134.8
February	April	8,787	2.2	876	5.9	10.0
February	June	2,920	14.3	1,035	14.9	2.8
March	April	873	1.9	21	1.9	41.6
March	June	849	6.2	19	5.5	44.7
April	June	138	12.0	17	11.0	8.1
Total						13.7

Research to Increase Bay Scallop Seed Resources

The deposition of swimming larvae in eddy zones with muddy bottoms combined with heavy predation predicts an area of low survival. Therefore, to increase the production of the bay scallop in its natural environment, it is most important to develop effective techniques for the collection of shelled larvae to support a program of marine farming. The new, bottom collecting method was developed and tested for this purpose.

Each collection bag in the new method had a total capacity of at least ten thousand larvae. A comparison of the new and the traditional hanging method of larval collection was performed (Table 6). The new method proved superior in all cases, collecting from 3 to 134 times the amount taken by the hanging method.

In order to increase the density of swimming bay scallop larvae in the field, we must increase the number of adult bay scallop shells. The fecundity of natural shell (90–100 mm shell length) is about 7,290,000 eggs per shell while shells from hanging cultures yield 27,529,000 eggs. Thus, the latter produces 3.78 times more eggs. To increase the number of spawned eggs in the field, we should therefore increase the numbers of hanging cultures. Thus, we can obtain the necessary numbers of swimming larvae from the cultured shells while using only one quarter to one third the number of natural shells that would otherwise be needed.

Conclusion

Increasing the deposited larvae does not always result in increased numbers of adult shell. The number of deposited larvae are affected by many factors. In order to obtain high survival rates when we release larvae collected from the natural environment, larval shell lengths should be greater than 30–50 m, and spat deposition sites should be low in organic matter and silt content and provide good substrates for byssus attachment.

Future research needs to focus on developing more effective methods for collecting juvenile shells and improving techniques to mark and track shells used in research.

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Release of Hormonally Sterilized Coho Salmon from a Puget Sound Hatchery

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ABSTRACT

The purpose of the study was to evaluate the use of hormonally sterilized fish in a fisheries enhancement project. Coho salmon (*Oncorhynchus kisutch*) at embryonic and fry stages were treated with 17α-methyltestosterone to prevent gonadal differentiation. The majority of the fish were reared at the Puyallup Salmon Hatchery, marked with coded wire tags and released as yearlings. For physiological studies, subgroups of the fish were transferred from the hatchery to the freshwater experimental hatchery in Seattle (NMFS), maintained for one year, and then transferred to seawater net pens. Gross examination of yearling fish in the treated group indicated 94% with undifferentiated gonads. Growth, development, and seawater survival of the sterile and control fish were generally equivalent. Analysis of data obtained from recoveries of released tagged fish in the fisheries revealed significant differences in the catch and distribution of control and sterile fish. As expected, control fish were caught in 1986 only, whereas sterile fish were caught in both 1986 and 1987. For 1986 and 1987 combined, the total catch of fish from the sterile groups was 33% lower than that for the control groups of fish. It is not clear from these data whether the reduced catch of sterile fish could be due to decreased marine survival or to reduced efficiency of catching the sterile fish. It can be concluded from these studies that the release of hormonally neutered fish from hatcheries results in an extended time for harvest of fish and slightly larger fish during the second year of harvest. However, the reduced numbers of sterile fish caught may not justify these benefits.

Introduction

Although sex of salmonids is normally determined by the genetics of the individual fish, steroid treatment of fish early in development can reverse or eliminate sex determination (*see* review by Hunter and Donaldson 1983). Treatment of salmon with appropriate doses of androgens during the time of normal sex differentiation can block this process and produce sterile fish. Since sterile salmon cannot reproduce, they will continue to live and grow beyond their normal age at reproduction.

There are many potential applications of sterile salmon to fisheries enhancement and aquaculture (Donaldson and Hunter 1982a). The elimination of sexual maturation and associated deterioration of flesh allows year-round marketing of adult salmon. Eliminating precocious maturation of males should increase the number of fish which reach harvestable size. Extending the age of the fish may allow the fish to grow to trophy-size. Since nonmaturing fish should not return to the hatchery, a portion of the smolts produced at the hatchery could, therefore, be specifically targeted for the fisheries.

Large numbers of hormonally sterilized coho salmon (*Oncorhynchus kisutch*) were first released from a Canadian hatchery in 1980 as part of a study by Donaldson and Hunter (1982b; Solar et al. 1986). The sterile fish did not return to the hatchery as did the normal control fish, but remained in the fishery for at least two additional years. Overall, the control fish contributed to the fishery at approximately twice the rate of the sterile fish. Although initial studies indicate that survival of hormonally sterilized salmonids in fresh water or in seawater net pens is not compromised, the question remains regarding the fishery contribution of such fish after their release into the environment. The present study was designed to evaluate the performance of hormonally sterilized coho salmon before and after release from a hatchery in the Puget Sound basin of Washington.

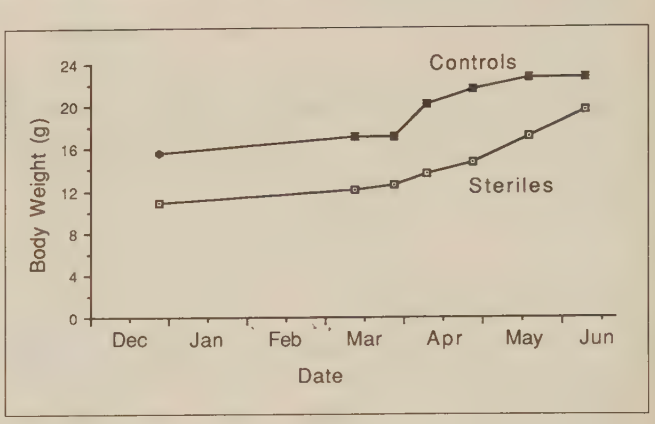

Figure 1
Average body weight of fish in control and hormone-treated groups maintained at the freshwater hatchery in Seattle from December 1984 to June 1985.

Methods and Materials

Coho salmon used in this study were from the normal production stock that returned to Puyallup Hatchery (Washington Department of Fisheries). Eggs were collected from the 1983 brood, fertilized, and maintained in incubation trays (Heath-Techna) which were supplied with water from Voight Creek (a tributary of the Puyallup River) at ambient temperature. Fish were subjected to steroid hormone treatment for sterilization similar to the method of Hunter et al. (1982). On 10 and 17 January 1984 when the embryos were well-eyed and on 8 and 15 March 1984 when the alevins were at approximately 50% yolk absorption, they were treated by immersion in a solution of 17α-methyltestosterone (MT; 300 μg/L) for two hours on each date. Approximately 90,000 fish in total received steroid treatment. When the fish were ponded, the control groups were placed in Burrough's raceways, and treated groups were placed in two rectangular fiberglass raceways (1 m × 1 m × 10 m). Starting with initial feeding and extending for 90 days, the experimental fish were fed a diet containing 20 mg MT per kg of diet. BioDiet (Bio Products, Warrenton, OR) was used as the starting feed; Oregon Moist Pellet (LaConner, WA) was used as the production feed. After termination of feeding with the treated diets, the experimental fish were placed in Burrough's raceways. The fish were maintained outdoors under natural photoperiod and temperature (approximate range 4° to 14°C).

Both control and experimental fish (approximately 120,000 total fish) were marked with coded wire nose-tags; tagged fish were identified by clipping the adipose fin. Both control and treated groups of fish were released as yearlings at the traditional release time (4 May 1985) and, in addition, at a delayed release time (30 June 1985). Each of the four release groups were replicated once, so that there was a total of eight tagging codes. All groups were released into Voight Creek.

In December 1984, 1,500 fish from both the experimental and control groups were transferred from Puyallup Hatchery to the freshwater hatchery facilities at the Northwest Fisheries Science Center (National Marine Fisheries Service) in Seattle. The fish were maintained outdoors in four cylindrical fiberglass tanks (1.3 m diameter × 1 m depth) supplied with dechlorinated municipal water at ambient temperature (5° to 16°C); the fish were fed Oregon Moist Pellets. The groups of fish (20 fish per group) in Seattle were weighed and sampled for blood at two-week intervals to determine thyroid and androgenic hormone levels during the parr to smolt transformation. On 15 May and on 18 July 1985, times which corresponded approximately to release times at the Puyallup Hatchery, groups of 150 fish from each group were transferred to floating net pens in seawater (27‰) at the National Marine Fisheries Service field station near Manchester, WA. The survival of the fish in seawater was determined for four months.

Blood plasma concentrations of thyroxine (T4) were determined according to Dickhoff et al. (1978); plasma levels of total androgens were measured according to Sower and Schreck (1982).

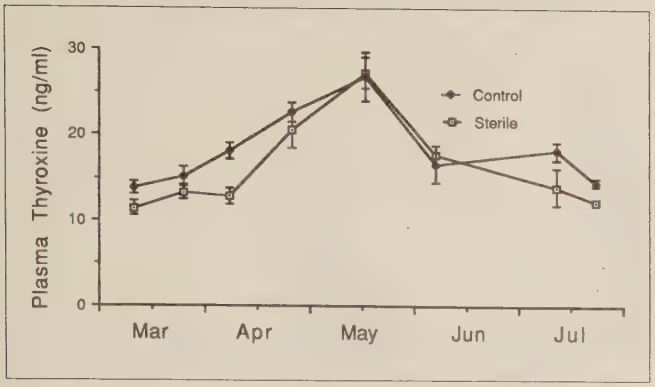

Figure 2
Blood plasma concentrations of thyroxine during the parr to smolt transformation (1985) of control and sterile fish maintained in the freshwater hatchery at Seattle. Symbols indicate means; brackets indicate plus or minus one standard error.

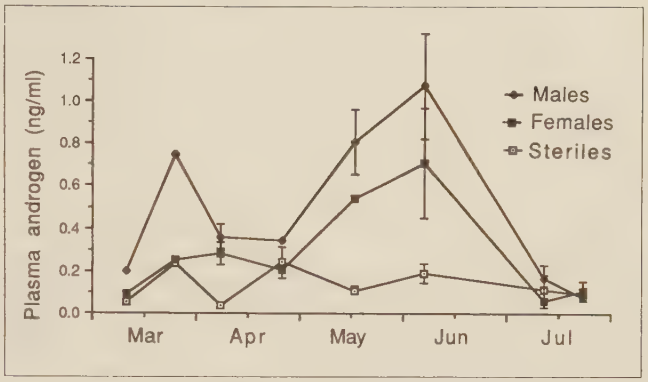

Figure 3
Blood plasma concentrations of total androgen during the parr to smolt transformation (1985) of control and sterile fish maintained in the freshwater hatchery at Seattle. Symbols indicate means; brackets indicate plus or minus one standard error.

Results

The body weights of fish sampled from the experimental and control groups are shown in Figure 1. The average body weight of the experimental group (steriles) was less than that of the control fish at the time the fish were obtained from the hatchery. The difference in average body weight between the two groups developed when the fish were maintained in the fiberglass raceways during the hormone treatment. During the sampling period, the steriles grew slightly faster than the controls so that their average body weight at the end of June was about 20% smaller. The ration of the fish at Puyallup Hatchery was controlled to produce equivalent-sized fish at the times of release. For the 4 May release, the average body weight of the control groups was 25.2 g and that of the steriles groups was 24.8 g. For the 30 June release both groups had average body weights of 37.8 g. Macroscopic analysis of the gonads of 160 yearling fish examined from the groups maintained in Seattle indicated 94% of the experimental group had no distinguishable gonads.

The plasma concentrations of thyroxine (T_4) during the parr to smolt transformation showed similar patterns for both the sterile and control fish held at the hatchery in Seattle (Fig. 2). During the early period of sampling, the mean level of T_4 was lower in the sterile compared to the control fish. However, both groups showed a peak in plasma T_4 in mid-May followed by a decline by the beginning of June. These data suggest that the timing of smoltification was equivalent in both groups, and that smoltification was completed by early June.

The blood plasma concentrations of total androgens showed variable patterns among the groups and throughout the sampling period (Fig. 3). The average androgen levels were highest in the male fish, intermediate in the females and lowest in the sterile fish. Plasma androgen levels were below the limit of detection in 25% of the sterile fish assayed. Androgen levels appeared to increase near the end of May and beginning of June in the male and female fish, but not in the sterile fish.

The survival of groups of fish transferred from the freshwater hatchery in Seattle to seawater net pens showed some slight differences between sterile and control groups (Fig. 4). In all groups initial mortality was low but mortality began to increase after one month in seawater. The sterile groups however, showed higher mortality compared to controls for both the May and July entries.

The fishery contribution of the control and experimental groups indicated that fish from both groups were caught in 1986, but only fish from the sterile groups were caught in significant numbers in 1987 (Fig. 5). However, the total recovery of released sterile fish was 15% in 1986, but only 0.3% in 1987. In both 1986 and 1987 the percent of fish caught from the May release groups was higher compared to the June release groups. In 1986 sterile fish contributed approximately 30% less than the controls. These data indicate that regardless of release time, the contribution of the sterile fish for both years was less than that of controls for one year.

The 1986 contribution of the four release groups to specific fisheries indicates that there were no major differences in the distribution of control and sterile fish (Fig. 6). As expected from previous information on the migration routes of this stock of fish, there was little contribution to the Oregon fisheries. The largest contribution was to the Puget Sound net fishery, followed by the British Columbia troll, British Columbia net, and Puget Sound sport fisheries. There was a slightly greater contribution of the sterile fish to the British Columbian troll and net fishery at the expense of the Puget Sound net fishery (Fig. 6).

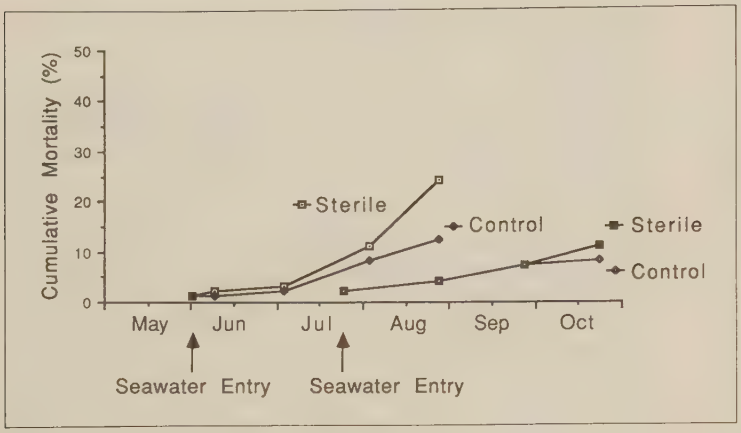

Figure 4
Cumulative seawater survival of control and sterile fish transferred from the freshwater hatchery in Seattle to net pens in seawater during 1985. Groups were transferred in May and in July.

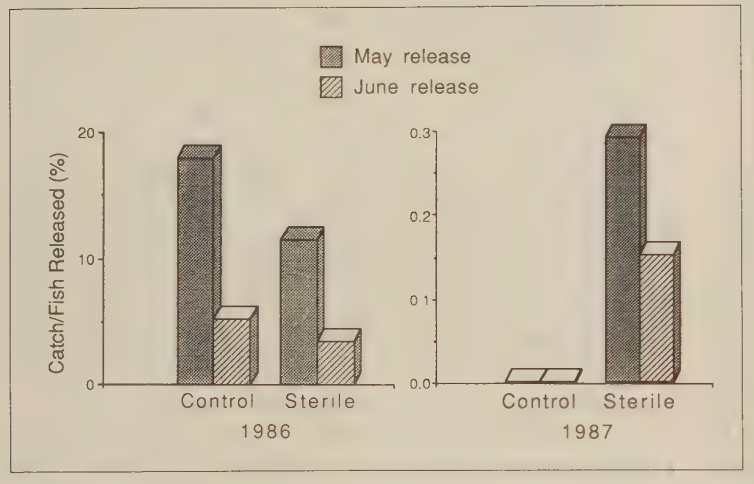

Figure 5
Contribution of control and sterile group of fish to the fishery during 1986 and 1987. Fish were released from the Puyallup Hatchery in May and June 1985. Bars indicate mean of replicated groups.

There was some return of the fish from the sterile groups to the hatchery. When the data are expressed as percent of total catch, a greater proportion of the sterile groups (14%) returned to the hatchery compared to the controls (8%).

Discussion

The fish from the androgen-treated groups (steriles) survived and contributed to the fisheries for at least one year beyond the normal time of adult maturation, as anticipated. However, the overall performance of the sterile groups of fish appeared to be poorer compared to controls; growth rate was slower, seawater survival was slightly reduced, and the fishery contribution showed a 30% reduction. The reason for the reduced performance of the sterile fish is not clear.

Previous studies on the release of sterile salmonids have also shown a reduced contribution or survival of the sterile fish. Coho salmon releases from the Capilano Hatchery in British Columbia indicated that the fishery contribution of the sterile group was only 45% that of the control fish (Solar et al. 1986). Releases of sterile coho and kokanee salmon (*O. nerka*) into a lacustrine environment suggested that survival or recapture of the sterile fish was markedly reduced (Parkinson and Tsumura 1988). Releases of sterile chum salmon (*O. keta*) from the west coast of Vancouver Island resulted in no recoveries of the sterile fish (E.M. Donaldson, Department of Fisheries and Oceans, West Vancouver, B.C. V7V 1N6, Canada, pers. commun., October 1988). Possible causes could be speculated for the reduced contribution of the sterile fish. Sterile fish may have reduced survival owing to poor physiological adaptability or reduced ability to avoid predators, among other possibilities. Alternatively, reduced contribution or recovery of sterile fish may be apparent because they are less vulnerable to capture gear. Furthermore, migration patterns of the sterile fish may differ from those of normal fish so that the steriles do not enter traditional fisheries.

Reduced growth or development of the sterile fish in either fresh water or seawater environments may result in reduced physiological adaptability. Our findings of slightly retarded growth and seawater adaptability support this

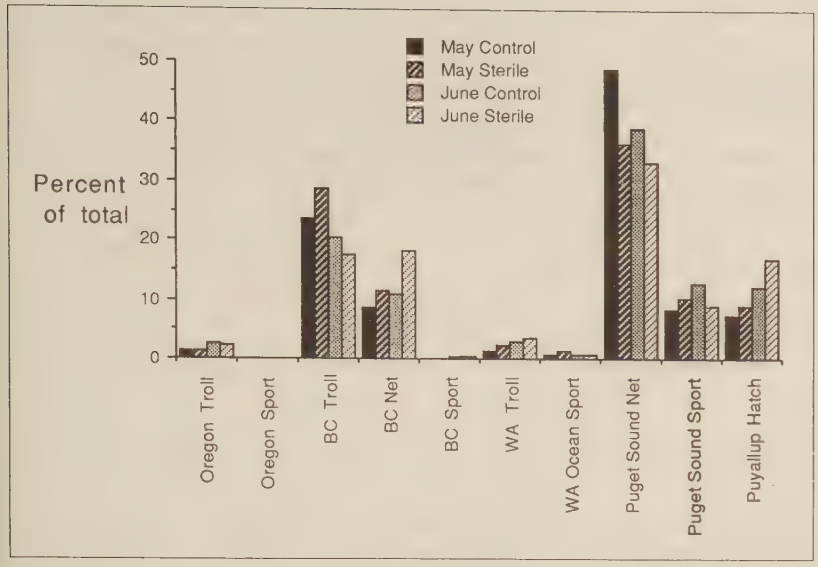

Figure 6
Percent of total catch in 1986 for each group of fish released from the Puyallup Hatchery. Bars indicate mean of replicated groups. Fisheries were separated as Oregon troll and sport; British Columbia (BC) troll, sport, and net; Washington (WA) ocean troll and sport; and Puget Sound net and sport fisheries.

hypothesis. The lowered levels of blood androgens may be one cause of reduced growth, since growth-promoting effects of androgenic hormones are well-established (Higgs et al. 1981). On the other hand, the pattern of thyroid hormones during smoltification and initial seawater survival of the sterile fish did not show significant differences compared to control fish.

The reduced level of androgens in the hormonally-sterilized fish suggests that not only the gametogenic activity but also the steroidogenic capacity of the gonadal tissue has been modified. The source of the measured circulating androgens in the sterile fish may be the interrenal gland. In addition to possible growth retardation, the reduced levels of androgens in the sterile fish may affect their behavior. Androgenic hormones contribute to aggressiveness, and such a behavioral trait may be important in food capture or other survival behaviors. Routes of out-migration or homing migration may be partly dependent on reproductive steroids, although our data do not indicate major differences in the geographic distribution of the fish as assessed by their appearance in specific fisheries.

It can be concluded from these studies that the release of hormonally neutered fish from hatcheries results in an extended time for harvest of fish. However, the reduced numbers of sterile fish caught may not justify this benefit. Our present findings, as well as the previously published results on releases of sterile salmonids, do not support widespread application of sterile salmon as a management tool for fisheries enhancement at the present time. Further research is needed to determine the causes of the reduction in contribution by the sterile fish. It is conceivable that possible developmental or physiological deficiencies of sterile fish may be compensated for by restoring the normal androgen levels in the fish.

Acknowledgments

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Large-Scale Culture System for Attaching Microalgae

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ABSTRACT

A stable microalgae foodsupply is necessary for the mass-production of marine animal seed to support marine farming in the coastal waters of Japan. Thus, a new apparatus for the mass-cultivation of attaching diatoms is described. Composed of a cylindrical glass vessel (diameter 10 cm, length 100 cm), nylon brush, and stainless steel rings, it is designed to maximize the surface area for diatom growth, and allow easy harvesting. After the initial inoculation of diatoms, filtered natural seawater is continuously flowed through the vessel under natural light conditions. A maximum of about 350 g of diatoms (wet weight) per month is produced with a harvest interval of three days. The diatoms harvested were useful as food for young sea cucumber and scallops. An attempt to culture monospecific diatom, using the modified apparatus and application of the apparatus to tertiary treatment of waste water, is also mentioned.

Introduction

It is becoming increasingly important to effectively utilize the coastal fisheries areas when we consider the changes occurring internationally—especially under the enactment by many countries of a "200 mile exclusive zone." In Japan, sea farming is considered a useful technology and is playing a greater role than ever before in the resource management of the coastal fisheries. Nowadays, various kinds of seed of marine animals are produced and liberated to natural habitats. In order to supply seed on a mega-scale, the stable mass-production of food organisms for these larval and young animals is necessary and has become a subject of primary importance.

"Attaching microalgae," particularly diatoms, are well known as an important food for young abalone, sea cucumbers, and sea urchins. Furthermore, it has been found that attaching diatoms promote the settlement and metamorphosis of marine animal larvae such as sea urchin, sea cucumber, and sea moss (Tani and Ito 1979; Z. Ikeda, Okayama Prefectural Fisheries Station, Ushimado, Oku, Okayama 701-43, Japan, pers. commun. August 1988; and Kitamura and Hirayama 1987, a and b; respectively). Tani and Ito (1979) examined the effects of attaching diatoms on the settlement and metamorphosis rate by using the pluteus larvae of sea urchin: They reported that larvae only settled on the bottom of tanks containing attaching diatoms, and that the metamorphosis rate to young sea urchin increased with the number of diatoms on the bottom.

Attaching diatoms have been cultured on the surface of polyvinylchloride or polycarbonate plates in tanks with flowing seawater. In the seed production system for abalone and other species, larval and young animals are usually reared together on the same plates. The balance between production and consumption of the algae plays an important role in the effective production of seed, and food deficiencies occur frequently owing to overcrowding.

In the present paper, the author describes a new apparatus for the mass-cultivation of attaching diatoms, and its various applications. The apparatus made it possible to isolate the process of algal culturing from the rearing of seed animals. The new source of harvested mass-produced cells may lead to a better technology for the seed production of useful marine animals.

Growth of Diatoms that attach in Natural Seawater

Attaching diatoms characteristically adhere to various living or nonliving substrata, taking up nutrients from the surrounding seawater. If sufficient amounts of fresh seawater are continuously supplied, they can even grow in seawater which is very poor in nutrients. Figure 1 shows the growth rate of *Nitzschia closterium* in flowing nutrient-

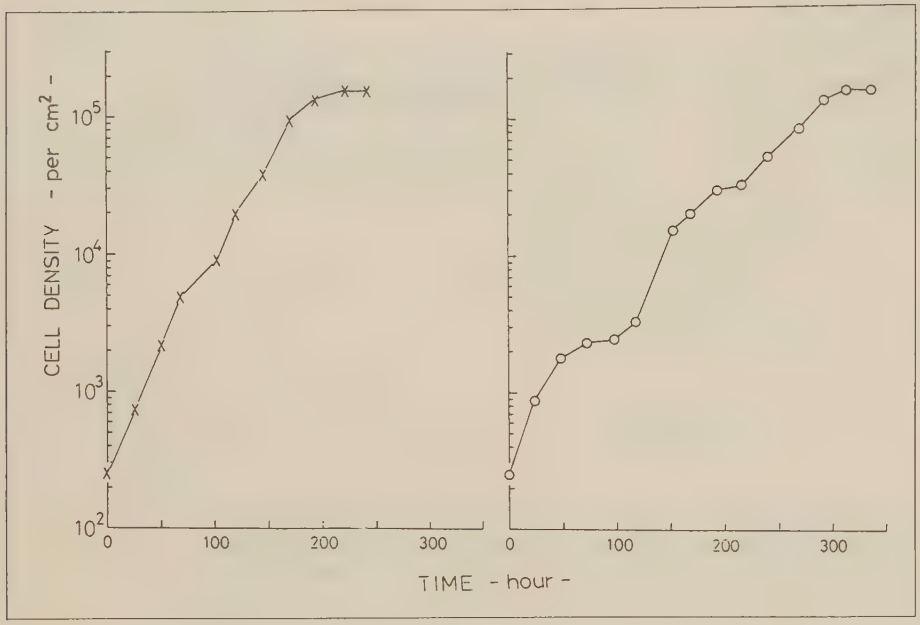

Figure 1
Growth curves of *Nitzschia closterium* in a batch of nutrient-enriched culture medium (**left**) and in running nutrient-poor natural seawater (**right**). After Tanaka (1984).

poor natural seawater (<10 μg atomic inorganic N/L and 1 μg atomic P/L as phosphate) comparable with that in a batch of the nutrient-enriched "Erd-Schreiber" culture medium (more than 1400 μg atomic inorganic N/L and 6 μg atomic P/L as phosphate) (Tanaka 1984, 1985).

The maximum yield of cultured diatoms is strongly dependent on the available surface area for cell attachment. Generally, maximum cell density is about 10^5–10^6 cells per cm^2 with excess cells seeming to scale off the substrata when overcrowding occurs.

Thus, the two factors, seawater flow rate and surface area for cell attachment, are of primary importance in the design of apparatus for the culture of attaching diatoms.

Cultivation Apparatus and Harvest of Cultured Diatoms

The apparatus, illustrated in Figure 2, is composed of a cylindrical glass vessel (diameter 10 cm, length 100 cm), nylon brush with stainless steel axis, and stainless steel rings. The nylon brush, also 10 cm and 100 cm, has 78,000 filaments, the diameter of each being 0.22 mm. Consequently, the surface area is about 3 m^2 per brush. The cultured diatoms attached to the filaments are harvested by moving the stainless steel rings up and down.

Approximately eight liters of a diatom suspension containing a few grams of diatoms (wet weight) was inoculated into the vessel. After thirty minutes, attachment of inoculated diatoms to the brush filaments was completed and sand-filtered natural seawater, which removes particles >50 μ/min diameter, was continuously flowed through the vessel from the lower side at the rate of at least 40 L/h.

During seven to ten days cultivation under natural light conditions, the diatoms proliferated and the nylon brush became dark brown in color because of the high density of cells. At this time the first harvest was made. A small portion of the diatoms always remained on the filaments after harvesting, thus re-inoculation was not necessary. Thereafter, cultured diatoms were harvested at an interval of several days. The relationship between the yield of the harvest and the period of harvest interval is illustrated in Figure 3. As calculated from the figure, the maximum yield per month was about 350 g wet weight with a harvest interval of three days. The data in Figure 3 was obtained from late autumn to early spring and, from the author's experiences, reflects expected yields the year round as well.

The composition of diatom harvests changed seasonally. However, the major species were always *Nitzschia* spp., *Navicula* spp., a *Pleurosigma* sp., and an *Amphora* sp. Tanaka (1987a) described the successional sequence of marine attaching biocommunities on fresh substrata. In general, attaching biocommunities are dominated in order by bacteria, motile solitary diatoms, nonmotile colonial diatoms and larger sessile plants and animals. The major diatoms cultured in the apparatus all belonged to the motile solitary diatom group occurring in the earliest stage of attaching diatom succession. The progress of diatom succession seems to be retarded with renewal of the brush surface after intermittent harvesting.

Under the continuous running of the apparatus, populations of filamentous green and blue-green algae gradually increased, which seemed to suppress the growth of diatoms. They were quite cohesive and were not removed by the up and down movement of the rings. Consequently the nylon brush needed to be replaced by a new one at

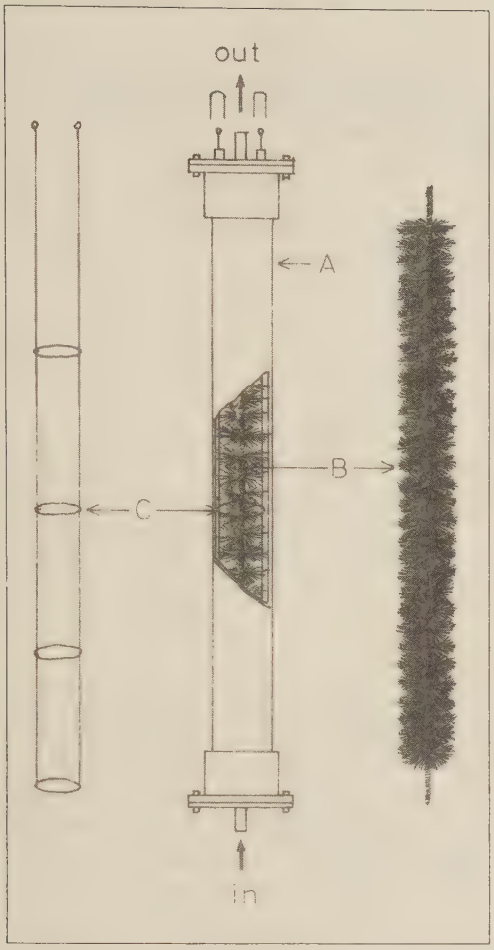

Figure 2
Schematic diagram of the mass-cultivation apparatus for attaching microalgae. (**A**) cylindrical glass vessel; (**B**) nylon brush; (**C**) stainless steel rings.

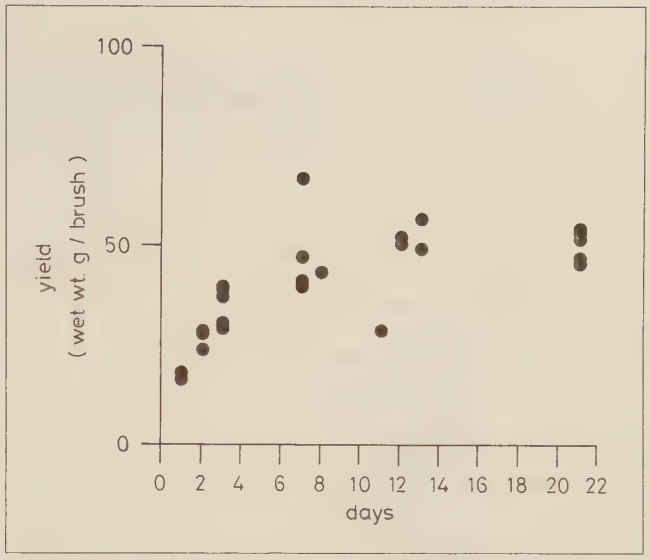

Figure 3
The relationship between the yield and the period of harvest interval.

Table 1
Rearing experiment of blueish type sea cucumber *Stichopus japonicus* Selenka fed with frozen attaching diatoms.

	Body (mm)	Body wet (mg)	Individuals	Survival (%)
Initial	1.7	0.3	62000	—
After 34 days	14.3	91.3	36000	58.5

an interval of three months in summer and four months in winter.

The harvested diatom cells formed suspended flocs, which were easily collected by sedimentation within several to ten minutes. These living diatoms can be used easily as food for abalone and other species because of their adhesive character which enables them to attach to substrata such as polyvinylchloride plates. The cells can be stored in a freezer after centrifugation and remain usable as food without appreciable decrease in performance (*see* discussion below).

Rearing of Young Sea Cucumber

Frozen attaching diatoms have been reported to be good food for young sea cucumbers by Yanagibashi et al. (1984). Ikeda and Kusaka of the Okayama Prefectual Fisheries Station also examined the food value of frozen attaching diatoms cultured by the author, using the present apparatus (Table 1; Z. Ikeda and K. Kusaka, Ushimado, Oku, Okayama 701-43, Japan, unpubl. data). In this study the average body length of sea cucumbers increased from 1.7 mm to 14.3 mm within only 34 days, much greater than the liberation size of 10 mm which is the adopted standard in Okayama Prefecture. The body weight increased from 0.3 mg to 91.3 mg. A total wet weight of 20 kg frozen diatoms was necessary for the production of 3.3 kg of sea cucumber seed. These results show that the cultured diatoms have a very high level of performance as food for sea cucumber. In addition, the supply of food is easily controlled by the separation of algal and seed production and a higher seed production efficiency results in comparison with the former method. When using the same polyvinylchloride plate for producing both diatoms and young sea cucumbers, it takes at least six months for seed to reach about 10 mm in length as opposed to approximately one month for the growth of an even larger seed with the new techniques.

Table 2

Species compositions of microalgae in the seawater and the contents of intestinal tract of bivalves at Gokasho Bay, Mie Prefecture (Tanaka 1987b). A: noble scallop *Chlamys nobilis* (Reeve); B: pearl oyster *Pinctada fucata* (Gould); C: oyster *Crassostrea gigas* (Thunberg); D: mussel *Mytilus edulis* Linne.

	Seawater contents (%) 1987			Intestinal contents (%) 10 Feb. 1987			
	1 Jan.	20 Feb	23 Mar	A	B	C	D
Planktonic species							
Peridinium sp.	3.2	1.3	17.8				
Gymnodinium nagasakiense			0.2				
Gyrodinium spp.	1.3		9.0				
Dinophysis acuminata	0.6		0.5	5.5	0.8		
Dictyocha fibula			0.5		0.8	1.1	
Polykrikos spp.	0.6		0.4				
Prorocentrum spp.	1.3		8.9				
Heterosigma akashiwo			0.1				
Chaetoceros spp.	65.1	92.9		12.1	31.5	26.1	14.8
Eucampia sp.	2.5	3.5	62.4	2.2		1.1	
Rhizosolenia sp.		2.2					
Skeletonema costatum	24.8					13.8	
Epiphytic species							
Cocconeis spp.				36.3	15.4	17.6	39.3
Nitzschia spp.				11.0	10.0	10.6	13.1
Navicula spp.				15.4	20.0	10.1	11.5
Coscinodiscus sp.				4.4	5.4	3.7	1.6
Gomphonema sp.				2.2	6.2	1.6	1.6
Others				11.0	10.0	14.3	18.0

Rearing of Young Scallops

The author has examined the intestinal contents of four species' bivalves taken from natural conditions (Table 2; Tanaka 1987b). These data do not necessarily reflect the exact compositions of food algae because of the destruction of naked flagellates and planktonic diatoms with thin silicic frustules in the intestinal tracts. However, attaching diatoms are undoubtedly an important part in the food composition of the bivalves' diet. In fact, attaching diatoms released from their substrata are observed in natural seawater, particularly in the innermost portion of Gokasho Bay, which is used as a shellfish culture ground (pers. observ.). Tanaka (1987b) also reported that the density of "drifting" attaching diatoms in seawater was 10^3–10^5 cells per liter all the year round in the innermost portions of Ago Bay (depth ≈2–5 m), Mie Prefecture, Japan.

In Japan, young or adult bivalves have scarcely been reared in tanks except during scientific experimentation. When rearing experimental bivalves, it is tedious for researchers to culture a large amount of food microalgae such as *Chaetoceros* and *Isochrysis*. If the diatom cells cultured using the present apparatus are useful as food for bivalves, the scientific study of bivalves will become less difficult.

The author examined the growth and survival of two kinds of young scallop (noble scallop *Chlamys nobilis* Reeve and Japanese scallop *Pecten albicans* Schröter) fed solely with attaching diatoms. Fresh diatoms were dissociated with an ultrasonic generator to each cell and dispersed in a tank through which sand-filtered seawater flowed (Tanaka 1988, unpubl. data). In the case of the noble scallop, the shell height and wet weight with shell increased from 23.0 ± 3.0 mm and 2.5 ± 0.9 g to 27.1 ± 3.7 mm and 3.4 ± 1.3 g, respectively, within 50 days rearing. The survival rate was high (97.1%) compared with that of the control experiment (29.0%) without feeding. In the case of Japanese scallop, the shell height and wet weight with shell also increased, from 19.8 ± 1.9 mm and 1.1 ± 0.3 g to 23.4 ± 2.1 mm and 1.8 ± 0.5 g, respectively, within the same time period. Although the growth of Japanese scallop was low, a high survival rate of 92.5% was obtained in the experimental groups, compared to only 2.5% in the control without feeding. These results show that cultured attaching diatoms are useful at least as supplementary food for both scallops.

Single-Species Culture

With the process of mass-culturing diatoms mentioned above, various kinds of unfavorable diatoms can easily contaminate the apparatus. The production of mono-

Table 3
Ratio (% cell number) of *Navicula ramosissima* in the harvest obtained by the large scale system for single species culture of attaching microalgae.

Date of harvest	Time (days)	*Navicula ramosissima*	Other diatoms[a]	Protozoa	Yield in wet wt. (g)
1987					
20 Nov	(0)	100.00	0.00	0.00	—
04 Dec.	(8)	100.00	0.00	0.00	5.06
16 Dec.	(20)	99.97	0.00	0.03	7.90
28 Dec.	(32)	99.95	0.01	0.04	37.10
1988					
05 Jan.	(40)	99.65	0.09	0.26	21.50
13 Jan.	(48)	94.65	3.82	1.53	38.50

[a]Predominantly *Nitzschia closterium*.

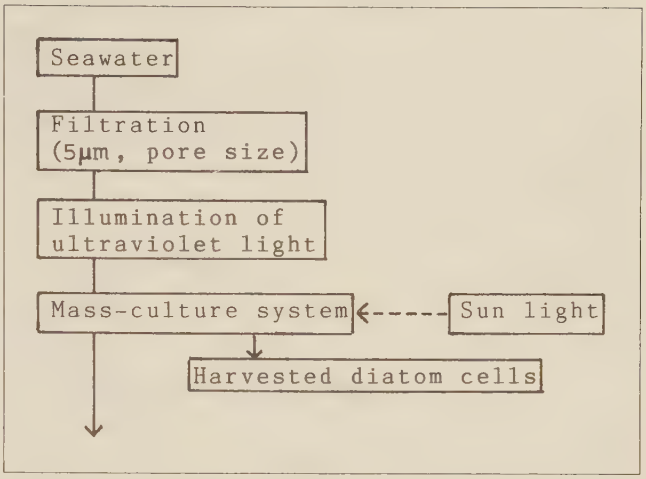

Figure 4
Methodology for unialgal cultivation.

species diatom cultures, having high growth rates and the maximum food value for a targeted marine animal, was desired. The author modified the present apparatus in an attempt to culture *Navicula ramosissima* (a strain isolated by Saga Prefectural Sea Farming Center) by the process illustrated in Figure 4. As shown in Table 3, the unialgal culture was maintained for forty days. The research for development of the unialgal mass-culture system is continuing and the final results will be published later.

Application to Waste Water Treatment

As mentioned earlier, attaching diatoms can proliferate in continuously supplied nutrient-poor fresh seawater and also in a nutrient-enriched medium with efficient use of nutrients. Therefore, the continuous flow system of the present apparatus can be easily applied to tertiary treatment of waste water. At the present time, the removal of inorganic nutrients from waste water after decomposition of organic matter is rather neglected in Japan.

The uptake rates of nutrient salts by attaching diatoms were examined by using a miniature version of the apparatus (Tanaka and Ohwada 1986). The diameter and length of the vessel were 5 cm and 50 cm, respectively. The brush had 21,000 filaments which were 0.20 mm in diameter, yielding a total of 0.33 m^2 surface area. The relationship between the uptake of nutrients and their concentration in seawater under the experimental conditions is illustrated in Figure 5 and 6. The results show that diatoms can efficiently remove the nutrients, particularly phosphate P (Fig. 6), from seawater. By harvesting diatoms from the apparatus at suitable intervals, diatoms would remain in their active growing phase and take up the nutrient salts from the flowing seawater.

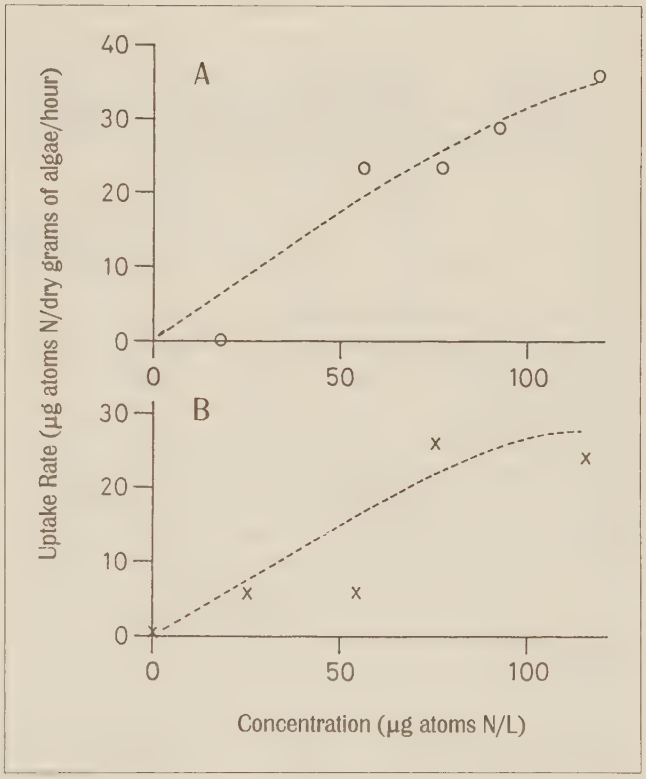

Figure 5
The relationship between diatoms uptake of nitrogen (g atom-equivalent) and nitrogen concentration in seawater. Flow rate is 2.41 L/h. (**A**) Nitrogen source is NO_3; (**B**) nitrogen source is NH_4.

There seems, however, to be many technical problems in the application of the apparatus to waste water treat-

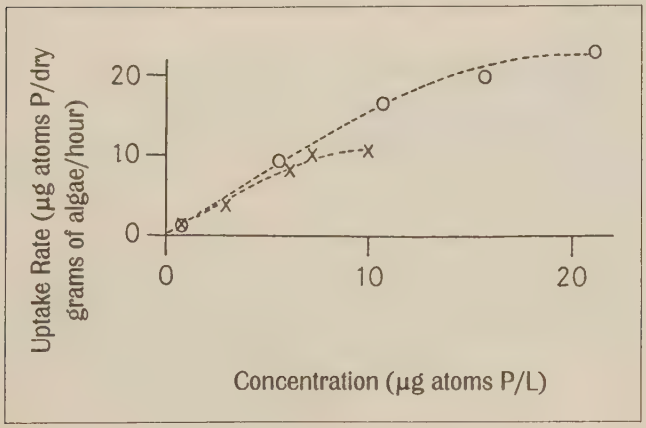

Figure 6

The relationship between diatom uptake of phosphorus (µg atom-equivalent) and phosphorus concentration in seawater. Flow rate is 2.41 L/h. X signifies that nitrogen source is NO_3; O signifies that nitrogen source is NH_4.

ment systems. For example, a portion of nitrate is reduced to nitrite by passing through the apparatus. Nitrite is toxic to various marine animals. More experimental work needs to be conducted to resolve these problems.

Acknowledgment

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The Importance of Smolt Development to Successful Marine Ranching of Pacific Salmon

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ABSTRACT

Fall run, underyearling chinook salmon (*Oncorhynchus tshawytscha*) released from four different hatcheries into the Columbia River system migrated faster as distance from the point of release to the estuary increased. Also, migrants traveling greater distances were more inclined to move to midriver by the time they reached the estuary. Fish released in various stages of smolt development, as measured by gill Na^+-K^+ ATPase activity, exhibited differences in migratory behavior and survival to adulthood. Those with more complete prerelease development a) migrated seaward more rapidly and were recovered at the upper entrance to the estuary in greater numbers than fish showing little or no smolting tendency at release, b) were recovered more frequently in midriver locations as opposed to nearshore areas for fish less well developed at release, and c) survived to adulthood in greater numbers. This study indicates that the degree of prerelease smolt development affects postrelease behavior and survival and is an important facet of the hatchery rearing period.

Introduction

Ultimately, the measure of success in the marine ranching of hatchery-reared salmonids is how many juveniles survive and contribute as adults. Although there are many environmental and developmental factors involved in determining survival or contribution, none of these can be designated most important all of the time. For example, one year a specific ocean condition may play a predominant role in determining survival, whereas another year the primary regulator of survival may be predation in the estuary, disease in the hatchery population, malnutrition, or any other of many factors. Investigators have proposed that survival of hatchery coho salmon (*Oncorhynchus kisutch*) in the northeastern Pacific Ocean off the coasts of northwestern United States and Canada is much better in years of strong upwelling (Gunsolus 1978; Nickelson 1986). Others have suggested that survival is density dependent (McGie 1981; McCarl and Rettig 1983) especially in years of low upwelling (McGie 1984; Peterman and Routledge 1983). Fisher and Pearcy (1988) found that high mortality of coho salmon juveniles occcurred within one month of ocean entry and that poor survival was not associated with starvation or low growth rates. They suggested that high predation may be responsible for low survivals and that this condition may be intensified in years of low upwelling. Hvidsten and Hansen (1988) reported increased numbers of adult Atlantic salmon (*Salmo salar* L.) recovered from hatchery smolts released during high water discharge and also concluded that predation during low discharge was responsible for decreased survival.

General health or condition of hatchery fish is known to influence ocean survival. High rearing densities adversely affected survival of coho salmon (Fagerlund et al. 1983; Sandercock and Stone 1982) and yearling spring chinook salmon (*O. tshawytscha*) (J.L. Banks, Abernathy Salmon Culture Technology Center, Longview, WA 98623, pers. commun., Nov. 1989). High rearing densities appear to affect smolt physiology by retarding development of increased plasma thyroxine levels, gill Na^+-K^+ ATPase activity and blood sodium regulatory ability (Schreck et al. 1985; Patino et al. 1986). Other hatchery conditions that create stress also affect the ability of the juvenile salmon to develop and function normally (Schreck 1982; Wedemeyer et al. 1984).

Few studies have looked at the relationship between smolt development in the hatchery and postrelease performance (e.g., seaward migration and survival to adulthood).

Among other changes, active seaward migration of smolts is accompanied by elevated levels of gill Na^+-K^+ ATPase activity (Zaugg and Wagner 1973; Bjornn et al. 1978; Schreck et al. 1980; Hart et al. 1981; Buckman and Ewing 1982; Zaugg 1982a; Weitkamp and Loeppke 1983; Rondorf et al. 1985, 1988; Zaugg et al. 1985; Rodgers et al. 1987), gill succinic dehydrogenase activity (Langdon and Thorpe 1985; Chernitsky 1986), and skin guanine content (Rodgers et al. 1987).

Greater adult contributions have been compared to higher gill Na^+-K^+ ATPase activity at release in coho salmon (Wahle and Zaugg 1982). Soivio and Virtanen (1985) reported that adult recapture rates were higher in hatchery-reared Atlantic salmon that had been

Table 1

Release dates and mean weights of underyearling fall chinook salmon from 15 Columbia River hatcheries (Vreeland 1990).

	Dates of release				Weight (g)			
Hatchery	1979	1980	1981	1982	1979	1980	1981	1982
Abernathy	4/17–5/18	4/09–5/14	4/15–5/26	4/20–6/01	4.8	7.7	6.6	8.9
Big Creek	5/21	5/13	5/07–5/18	5/17	5.6	5.8	5.9	6.1
Bonneville	5/01–5/29	5/20	4/24	4/23	6.1	6.1	6.2	5.7
Cowlitz	6/27–10/16	6/03–7/11	6/27–6/28	6/24–7/08	5.3	3.5	5.3	5.0
Elokomin	6/15	6/19	6/01	6/15	4.6	5.7	4.5	5.7
Grays River	6/09–6/12	6/01–6/24	6/01–6/08	6/01	4.9	5.3	5.3	5.2
Kalama Falls	6/22–7/13	6/13–6/24	5/22–5/28	6/10–7/02	2.6	3.7	4.4	4.5
Klaskanine	5/29	6/04	5/10	6/07	6.4	5.7	5.3	5.3
Klickitat	5/14–6/13	5/27	6/05	6/04	5.7	5.3	5.8	5.5
Little White	6/22	6/10	6/04–6/05	6/02–6/03	4.1	4.5	4.8	4.9
Priest Rapids	5/23	5/20–6/24	6/23–6/24	5/24–6/16	6.1	6.6	5.1	5.2
Sea Resources	5/01–5/31	5/20	4/16–4/29	4/01–5/07	4.1	5.0	4.6	4.5
Stayton Pond	5/07–5/21	4/20–5/21	4/27–6/16	5/03–5/21	6.8	5.2	6.1	5.2
Washougal	6/14–9/02	6/30	6/30–7/06	6/30–7/06	5.8	4.6	6.1	5.0
Spring Creek	3/20–5/18	3/10–5/09	3/25–5/05	3/25–5/21	5.7	6.0	5.2	6.2

Table 2

Percent adult recovery (Vreeland 1990) and between-year rank order for fall chinook salmon released as underyearlings from 15 Columbia River hatcheries. Years are ranked 1 to 4 in descending order of percent recovery of adults (commercial and sport fishery, and hatchery returns) from juveniles released in the year shown.

	Percent recovery				Rank order (year of release)			
Hatchery	1979	1980	1981	1982	1979	1980	1981	1982
Abernathy	0.56	0.58	1.00	0.20	3	2	1	4
Big Creek	0.38	1.04	0.27	0.54	3	1	4	2
Bonneville	0.38	0.14	0.27	0.31	1	4	3	2
Cowlitz	0.28	0.23	0.40	0.17	2	3	1	4
Elokomin	0.01	0.08	0.11	0.03	4	2	1	3
Grays River	0.06	0.20	0.23	0.04	3	2	1	4
Kalama Falls	0.06	0.29	0.16	0.18	4	1	3	2
Klaskanine	0.13	0.14	0.07	0.05	2	1	3	4
Klickitat	0.11	0.16	0.04	0.10	2	1	4	3
Little White	0.20	0.30	0.27	0.019	3	1	2	4
Priest Rapids	0.17	0.42	0.54	0.57	4	3	2	1
Sea Resources	0.10	0.40	0.14	0.70	4	2	3	1
Stayton Pond	0.75	0.74	0.32	0.45	1	2	4	3
Washougal	0.13	0.33	0.21	0.19	4	1	2	3
Spring Creek	1.01	1.47	0.40	0.43	2	1	4	3
Total numbers for each rank order: 1's:					2	7	4	2
2's:					4	5	3	3
3's:					4	2	4	5
4's:					5	1	4	5

judged to be better smolts using a series of physiological indices.

This report 1) examines in- and between-year variations in adult survival of fall chinook salmon released as underyearlings from fifteen Columbia River Basin hatcheries, 2) looks at the influence of migration distance on rate of migration and horizontal position of juvenile migrants as they approach the estuary and, 3) reports adult returns from juveniles released during five successive years at one specific hatchery in comparison to degree of smolt development as measured by pre-release gill Na^+-K^+ ATPase activity and by post-release migratory behavior.

Materials and Methods

All fish used for gill ATPase determinations were underyearling fall chinook salmon. Fish were taken randomly from production ponds (biweekly) by dip net, killed by a blow to the head, weighed (g), and measured (fork length, mm). Gill filaments were trimmed from the arches, placed in buffered sucrose, and stored at $-25°C$ until analysis of Na^+-K^+ ATPase activity (μmoles $P_i \cdot$ mg protein$^{-1} \cdot h^{-1}$) as described by Zaugg (1982b). On each sampling date during the period 1978–80, samples of gill filaments were taken from 30 fish. These were divided into 10 assay samples, each containing gill filaments from 3 fish. In 1981 and 1982, 10 to 40 fish were taken on each sampling date and used for individual ATPase analysis.

Migrants were captured daily in the Columbia River at Jones Beach, Oregon, 75 km upriver from the mouth and identified from coded wire tags. Captures were accomplished nearshore by seining from the beach with a 95-m seine and in deep-water, midriver channels using a purse seine (Dawley et al. 1985). Numbers of migrants caught were adjusted to a standard fishing effort. Migration rates were estimated by dividing the distance from release points to the recovery point at Jones Beach by the number of days from the first day of release to the date of median fish recovery. In 1979 underyearling chinook salmon with coded wire tags (some groups in replicate) were released at approximately the same time in June from four hatcheries located at varying distances from Jones Beach. Migrants were captured at Jones Beach with purse and beach seines as described earlier. Adult recovery information was obtained from the Pacific Marine Fishery Commission, Portland, Oregon, and from Vreeland (1990). Adult recoveries were estimated from samples of the commercial and sport fishery catches and hatchery returns.

Results

In a 4-year study described by Vreeland (1990), fall chinook salmon released as underyearlings from 15 Columbia River hatcheries weighed less than 10 g (Table 1). Although annual differences in weight were not great at any given hatchery there were significant differences between hatcheries. Usually, each hatchery released about the same time of the year for each year of the study. Percent total adult recoveries and between-year rank orders for each hatchery are shown in Table 2. Most rank orders were ones and twos, and fewest were threes and fours observed for adult recoveries of fish released in 1980. Nevertheless, the general random order of rank indicates that there is much between-year variability in total adult recoveries among the groups of fish released from the 15 hatcheries during the four-year period.

Underyearling chinook salmon were released from four Columbia River Basin hatcheries in 1979, presumably in about the same stage of development, prior to any increase in gill Na^+-K^+ ATPase activity (Table 3). Migrants captured at Jones Beach showed that those fish released at greater distances traveled downstream at higher rates

Table 3

Changes in migrations rates with distance from point of release for tagged underyearling chinook salmon released from hatcheries and caught at Jones Beach.

	At release				Migration distance (km)	Caught at Jones Beach	
Hatchery	Date: June1979	Wt. (g)	Number tagged	Gill ATPase		Beach seine	Purse seine
Kalama Falls	30	2.5	214,500	7	66	2,799	206
Toutle	17	2.8	12,000	7	85	85	11
Toutle	17	2.8	132,000	7	85	973	108
Washougal	14	4.8	93,700	9	138	318	43
Washougal	14	4.8	154,500	9	138	634	104
Little White	22	4.3	177,800	10	186	221	131
Little White	22	3.7	264,800	10	186	400	161

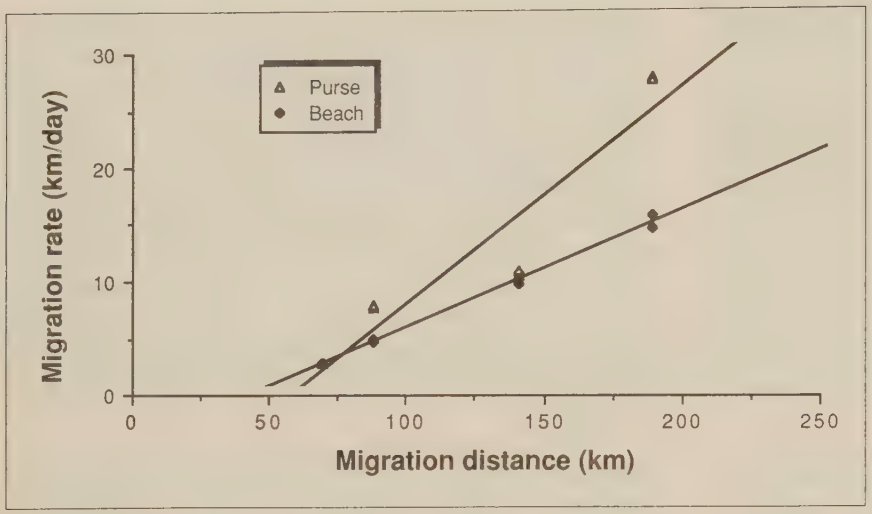

Figure 1
Increase of median migration rate with distance traveled by underyearling chinook salmon. Seven tagged groups were released from 4 hatcheries during 14–30 June 1979 (Table 3) and migrants were captured at Jones Beach with a beach or purse seine. Correlation coefficients for migration rates vs. distance are purse, 0.94 ($P<0.01$); beach, 0.99 ($P<0.01$). Data from Dawley et al. 1985.

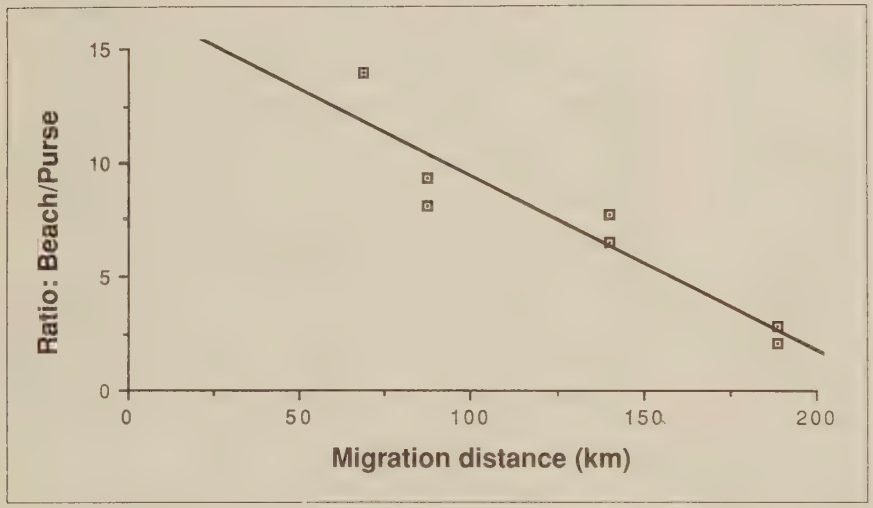

Figure 2
Ratio of the number of underyearling chinook salmon caught in the beach seine to the number of those caught in the purse seine at Jones Beach vs. distance migrated, 14–30 June 1979. Correlation coefficient 0.93 ($P<0.01$).

(Fig. 1). Those captured in midriver with the purse seine traveled faster than those captured near shore with the beach seine (Fig. 1). In addition, fish traveling farther were caught in increasing numbers in the midriver purse seine (Fig. 2), indicating movement from shoreline to midriver migration with time and with distance from release. These observations are summarized in Figure 3.

Figure 4 presents an idealized profile for gill Na^+-K^+ ATPase activity generally observed in hatchery populations of anadromous salmonids during smolt development, when fish are either held for extended periods in the hatchery environment or released to migrate seaward. The shaded portion under the profile represents that percent of the total area under the curve that is completed by the time of a hypothetical release. We have used this percent as a basis to evaluate the relationship between gill Na^+-K^+ ATPase activity in groups of underyearling chinook salmon released from the Spring Creek National Fish hatchery during 1978 through 1982, and post release performance, as reflected in migratory behavior and survival to adulthood (Fig. 5). This percentage was compared to the numbers of smolts captured at Jones Beach (migration distance = 194 km) and to adult recoveries from fish released in each of those years (Table 4). Correlations and significance of the data collected during the study are also shown in Table 4.

Discussion

The four-year study of underyearling fall chinook salmon released from hatcheries in the Columbia River Basin reported by Vreeland (1990) illustrates that considerable variability in adult recoveries exists from year to year among the several production facilities. Rank order information suggests that fish released from most of the hatcheries in 1980 performed better than fish released in other years. It would appear, therefore, that conditions in the estuary and ocean were more favorable for fish released in 1980 than those for fish released in the other three years of the study. Fisher and Pearcy (1988) have shown that

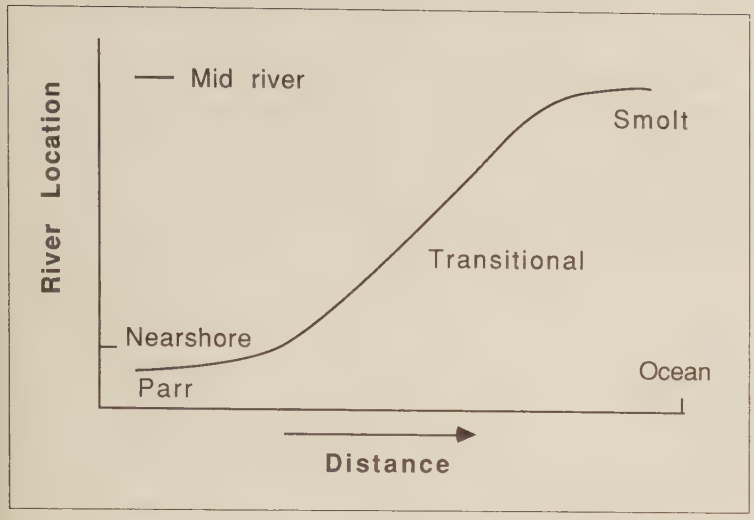

Figure 3
Summary of expected migratory behavior for underyearling fall chinook salmon released into the Columbia River system and completing smolt transformation during migration.

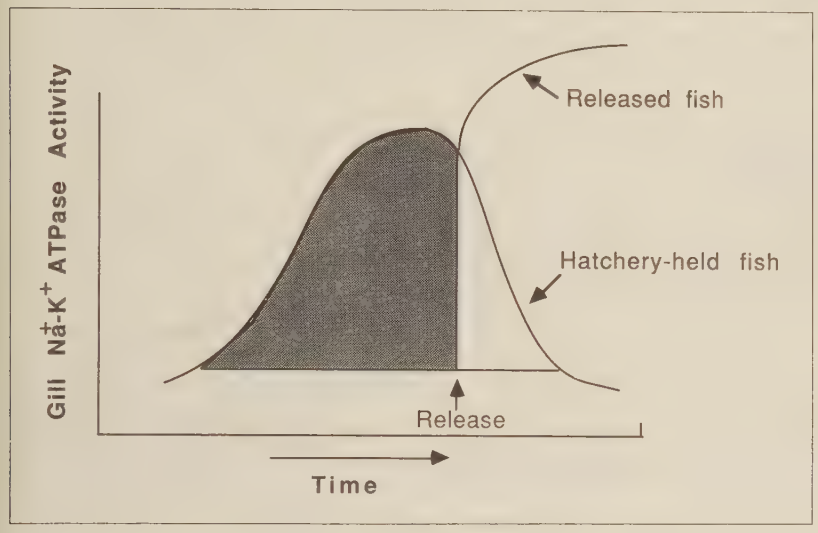

Figure 4
Illustration of a typical gill Na^+-K^+ ATPase activity profile for hatchery-reared anadromous salmonids undergoing parr-smolt transformation and either retained at the hatchery or released to migrate seaward. The shaded area represents that portion of the total area under the curve completed by the time of a hypothetical release.

the success of any year class of coho salmon may be determined soon after ocean entry, and this may also be the critical time for juvenile chinook salmon. If so, the degree of smolt development at that time may be a significant contributing factor to survival.

Nevertheless, among the 15 hatcheries shown here, the patterns of adult recovery varied widely in an inconsistent manner. For example, adult recoveries from the Little White Salmon hatchery (river km 261) ranged from only 0.02 to 0.03 percent during the four years of the study whereas recoveries from the Spring Creek hatchery (located only 9 km further upstream) were 50-fold greater. Fish released from the Bonneville hatchery (31 km downstream) had adult recoveries that were intermediate between the other two hatcheries during the initial two years (1979–80) and similar to Spring Creek for the final two years (1981–82). These observations strongly suggest that hatchery rearing methods and practices greatly influence survival to adulthood.

Variability in gill Na^+-K^+ ATPase activity profiles in chinook salmon reared at the Spring Creek hatchery from 1978 to 1982 is further evidence that physiological development in hatchery-held salmon can differ from one year to the next. Important, but subtle differences in rearing methods and diets may have greater influences on juvenile development and survival than is commonly thought.

Dawley et al. (1986) reported that migration rates of underyearling chinook salmon in the Columbia River increased with distance traveled. However, these investigators did not assess possible differences in degree of smolt development in the fish used for their study. The degree of smolt development reached in steelhead (*O. mykiss*) at the time of release affected rates of migration (Zaugg and Wagner 1973). As the act of migration proceeds, smolt development is intensified (Zaugg 1982a; Zaugg et al. 1985; Rondorf et al. 1985, 1988). In the present study fish released 186 km from Jones Beach (Little White Salmon hatchery) probably underwent more extensive

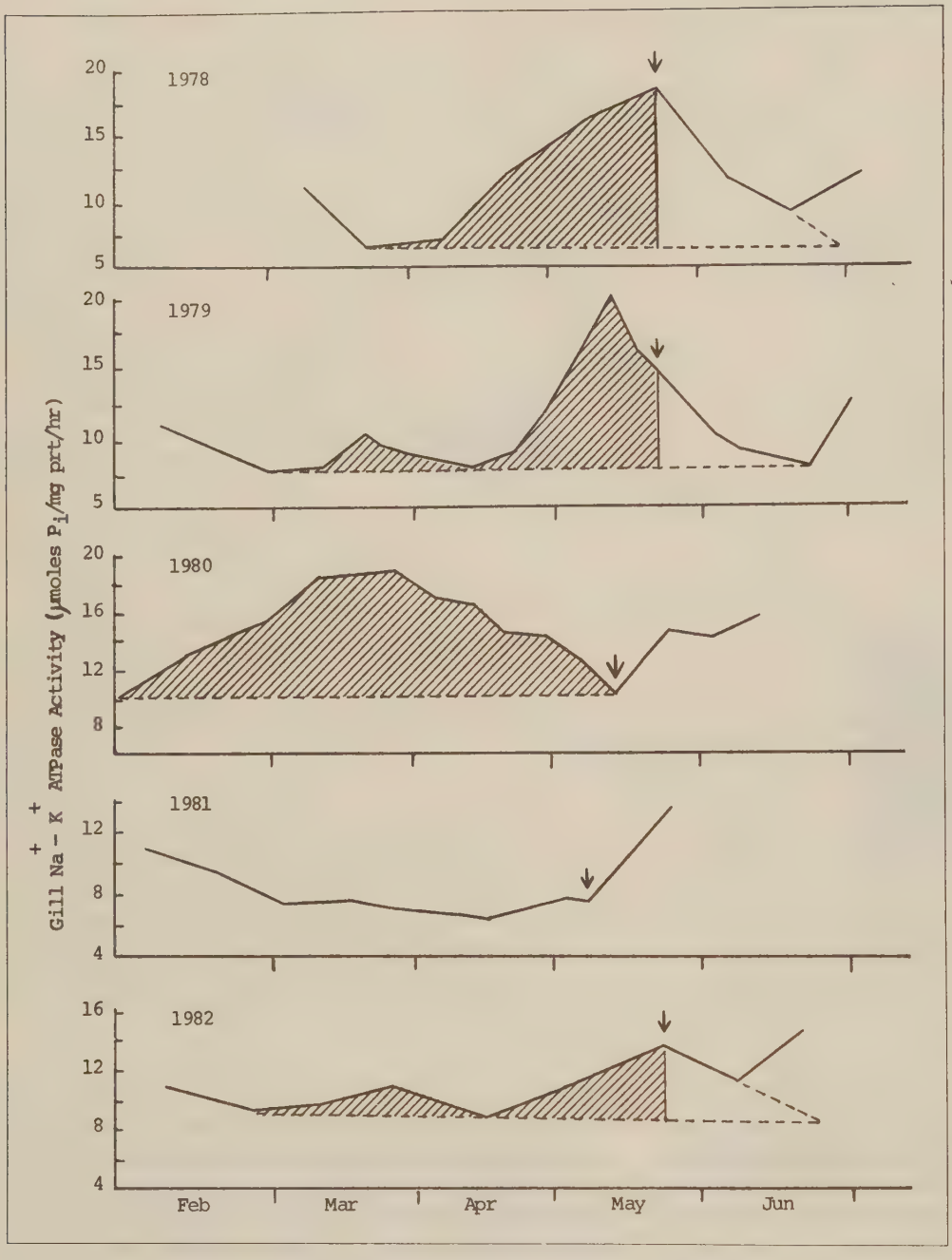

Figure 5
Gill Na^+-K^+ ATPase activity profiles for underyearling fall chinook salmon at Spring Creek National Fish Hatchery, 1978–82. Arrows indicate the date of final release (groups were also released in March and April). The shaded areas represent that portion of the total area under the curve (dashed lines) completed by the final release dates. Spring Creek hatchery is located at river km 269 on the Washington shore of the Columbia River. prt = protein.

smolt development during migration and thus exhibited more rapid downstream movement over the total distance traveled than did fish released closer to the capture site (Kalama Falls, Toutle, and Washougal hatcheries). It appears that more completely smolted fish move offshore to faster-moving midriver waters as indicated by both faster migration rates and higher percentages of migrants being caught in the midriver purse seine. The ratio of numbers caught in the beach seine to numbers caught in the purse seine decreased as the distance traveled increased.

Generally, underyearling chinook salmon, as well as yearling coho salmon, migrate more rapidly when released while gill Na^+-K^+ ATPase activity is declining (following a previous increase and peak) than when the activity is on the increase (before peaking, Zaugg 1982a). When releases are made during the period of decline, however, ATPase activities are rapidly re-elevated as migrants move downriver. Ewing et al. (1984) showed that underyearling spring chinook salmon released later (mid-June vs. mid-May) migrated faster and in equal numbers. However, when released in August these

Table 4

Comparisons of shaded areas under ATPase profiles (expressed as percent of the total area under the curve, Fig. 5) to various measurements of post-release performance of all fall chinook salmon released (March, April, and May each year) from Spring Creek National Fish Hatchery[a].

Year of release[b]	Percent ATPase curve[c]	Percent caught at Jones Beach[d]	Percent caught in purse seine[e]	Ratio beach/purse seine[f]	Percent adult recovery[g]
1978	65	0.14	0.018	6.9	0.87
1979	80	0.17	0.036	3.6	1.01
1980	100	0.20	0.031	5.3	1.47
1981	0	0.12	0.006	18.1	0.40
1982	66	0.14	0.009	14.1	0.43
Correlation coefficients and significance					
% ATPase	—	0.86	0.77	0.84	0.83
		$P<0.1$	$P<0.2$	$P<0.1$	$P<0.1$
% Caught (total)	—	—	0.86	0.78	0.93
			$P<0.1$	$P<0.1$	$P<0.2$
% Caught (purse)	—	—	—	0.93	0.87
				$P<0.02$	$P<0.05$
Ratio	—	—	—	—	0.85
					$P<0.05$

[a] Spring Creek National Fish Hatchery is located at river km 269 on the Columbia River.
[b] Three releases were made each year, in March, April, and May.
[c] Percent of the total area under profiles in Figure 5, depicted by shading.
[d] Percent of all fish released (March, April, and May) caught at Jones Beach (rkm 75) with both beach and purse seines. Numbers adjusted to a standard fishing effort (Dawley et al. 1985).
[e] Percent of all fish released (March, April, and May) caught at Jones Beach (rkm 75) in the mid-river purse seine. Numbers adjusted to a standard fishing effort (Dawley et al. 1985).
[f] Ratio of numbers of migrants caught at Jones Beach in the beach seine to numbers caught in the purse seine for all fish released each year (March, April, and May).
[g] Includes adults caught in the commercial and sport fishery, and returns to the Spring Creek hatchery from all three releases each year. Data from Pacific Marine Fishery Commission, Portland, OR and Vreeland (1990).

salmon showed reduced migration and an increase in stream residence.

The importance of elevated gill Na^+-K^+ ATPase activity for successful adaptation to seawater has been demonstrated in hatchery- and laboratory-reared steelhead (Adams et al. 1975), coho salmon (Harache et al. 1980), and Atlantic salmon (McCormick et al. 1987; Besner and Audet 1988). Observations suggest that smolts actively migrating seaward in the natural environment also require high gill Na^+-K^+ ATPase activities for successful transition to seawater.

We have attempted to determine whether smolting-associated pre-release increases in gill Na^+-K^+ ATPase activity are related to post-release behavior and survival of underyearling chinook salmon. In order to relate the enzyme activity quantitatively to post-release behavior and survival we have used as a measurement that portion of the total area under the ATPase profile curve which is completed by release time. This method takes into account post-release migrational characteristics that appear to be more influenced by the time-dependent development pattern of the enzyme activity than by the absolute value at the highest point. By applying this analysis to the gill Na^+-K^+ ATPase activity profiles observed for five successive years in underyearling fall chinook salmon at the Spring Creek hatchery, we obtained results which support assumptions that the degree of smolt development is related to the numbers of migrants reaching the estuary, their location in the river, and to percent adult recovery. Although the percent ATPase completed by the May release of 1982 is similar to the percent in 1978 (66 and 65), other observations for that year (migrants caught in the purse seine, beach/purse seine ratios, and adult recovery) more closely resemble the values obtained in 1981. The total area under the 1982 profile is small compared with the years of better survival (1978–80) and it appears that this may also indicate poor smolt development during the hatchery rearing period.

If the degree of smolt development reached by the time of release is important to survival, and that degree of

development is inconsistent from one year to the next, as seen at the Spring Creek hatchery, then it becomes important to identify and control as many of the variables involved as possible. Although factors causing variable smolt development in the Spring Creek fish during the five-year study remain unidentified, there are known culture practices and conditions that are candidates. Ogata and Konno (1986) demonstrated that additional dietary lipid may promote smolt development in cherry salmon (*O. masou*). Other dietary ingredients undoubtedly influence smolt development and may need closer scrutiny to insure more uniform smolting patterns. Growth rate, temperature, and size are interdependent and can affect the rate and extent of smolt development. Although better adult returns are generally associated with larger juveniles there was no significant relationship between size at or time of release and adult recovery in the Spring Creek study (Zaugg 1989). Disease and disease treatments, pond densities, and stresses accompanying normal and abnormal hatchery operations must certainly cause year-to-year variablility in the ability of hatchery-reared fish to develop properly. These and other, perhaps yet undetermined factors, must surely be important to the success of a hatchery program.

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Control of Skin Ulcers in Young Bluefin Tuna in Fish Farming

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ABSTRACT

Pathologial, bacteriological, and chemotherapeutic studies were carried out to clarify the mechanism of skin ulcer formation and to control the disease in young bluefin tuna (*Thunnus thynnus*). The structure of bluefin tuna skin is principally the same as that of other fishes; however, the epidermal layer is very thin and fragile, and handling promotes the development of skin ulcers. Histopathology revealed various inflammatory reactions and an increase in the bacterial content within the ulcer foci. Bacteriological examinations showed that *Vibrio*, which is not ordinarily isolated from normal skin, became dominant in the infected area. The fact that the *Vibrio* was also isolated from the kidneys of fish with advanced skin ulcers indicates that the ulcer foci were a possible entrance for bacteria into the body. Study on the responses of the bacteria isolated from the skin ulcer to various antimicrobial agents indicated that *Vibrio* was specifically sensitive to sodium nifurstyrenate (NFS-Na). A bath treatment with NFS-Na decreased the mortality of fish suffering from skin ulcers formed as a result of their transportation by boat for examination.

Introduction

Bluefin tuna, *Thunnus thynnus*, is one of the most important commercial species in Japan. It is well known that young bluefin tuna are very fragile and handling results in high mortality (Ueyanagi et al. 1973). Almost all the fish captured show severe skin sulcers and die. A large portion of the mortality during captivity is caused by or at least related to the skin ulcer. Thus, it is very important to control the skin ulcer to promote the bluefin tuna ranching program.

This paper describes pathological, bacteriological, and chemotherapeutic studies carried out to clarify the mechanism of skin ulcer development and to lead to the development of control measures against the disease.

Materials and Methods

Experimental Fish

The fish used in the present experiments were young bluefin tuna captured by surface trolling in the offshore waters of Kaminokae, Nakatosa-cho, Kochi Prefecture from July to August 1981–85. The tuna were kept in floating net cages (4 × 4 × 4 m) settled in Kaminokae Bay and were fed sand lances (*Ammodytes personatus*). In some experiments they were transported to our laboratory in Nansei-cho, Mie Prefecture by fishing boat in a live well (1.5 × 1.5 × 0.8 m), and reared in round water tanks (diameter 4.3 m, depth 0.8 m, net vol. 8,000 L) on land, with continuously supplied seawater. These laboratory-reared fish were also fed sand lances. The fish were transferred from boats to pens directly with a hand net. However, from the transportation boat to land tanks, they were transferred into 20-L containers (1 fish at a time) with a hand net and then transferred to the tanks.

Pathological Observations of Skin Ulcers

For pathological observation of the ulcers, various stages of ulcer foci were sampled. They were fixed in Bouin's solution for 10–24 hours, embedded in paraffin and sectioned at 5 μm. The sections were stained with either hematoxylin-eosin or Giemsa.

Bacteriological Examinations of Skin Ulcers

Skin ulcers were produced experimentally by gently seizing fish at the caudal peduncle with a cotton glove. Usually a severe ulcer developed on the portion of the skin contacted by the glove. Ulcers were induced in twenty young fish ranging from 55 to 90 g by handling them; three fish were used for each of the bacteriological examinations performed at 0 and 24 hours after contact with the skin. The fish were reared in the pen. The water temperature ranged from 25 to 30°C during this experiment.

The number of bacteria in the ulcer foci of the skin was determined by the spread plate technique. A portion (2 × 1 cm area) of skin was excised aseptically and homogenized with 10 mL of saline solution (0.8% NaCl and 1.0% KCl). After three 10-fold dilutions of the homogenate, 0.1 mL of each was spread on the duplicate plates of B medium (Simidu and Hasuo 1968). After incubation at 25–30°C for 5 days, the colonies were counted. Bacterial colonies were collected randomly and used for the identification of bacteria. For the identification of bacteria, Gram staining, cell form, motility, catalase, cytochrome oxidase, pigment production, OF and gas production from glucose were tested. To determine the invasion of bacteria into the internal organs, a loopful of blood from the kidney was streaked on B medium.

Chemotherapy of Skin Ulcers by Sodium Nifurstyrenate

Three hundred young bluefin tuna (70–200 g) were transported by fishing boat from the Kaminokae pens to our laboratory. The trip took about 24 hours. Just after arrival they were divided into 2 groups, each kept in separate tanks (8,000 L) as previously described. Sodium nifurstyrenate (NFS-Na) was dissolved in the seawater of one tank to a concentration of 5 ppm for 30 minutes once a day for two days, while no treatment was done in the other tank. Each group was reared with the same water supply which ranged in temperature from 25 to 28°C and were fed sand lances daily. Mortalities of the two groups were compared after 11 days.

To study the effect of NFS-Na on the bacteria found in the skin ulcers, infections were produced artificially in 30 young bluefin tuna with a cotton glove. They were divided into 2 groups each kept in separate tanks (8,000 L) as previously described. In one tank NFS-Na was dissolved in the rearing seawater to a concentration of 5 ppm for 30 minutes once a day for two days: the first treatment was done just after handling, and the second one was done just after 24 hours sampling. No treatment was done on the other group. Two fish were removed for bacterial examination from each group 2, 24, and 48 hours after the contact with the skin. The water temperature ranged from 25 to 26°C during this experiment. Methods for counting the number of bacteria and for the identification of bacterial flora in the skin and kidney were principally the same as those mentioned above, although seawater and ZoBell 2216E medium were used as the diluent and culture medium. Responses of the isolates to NFS-Na, chloramphenicol (CP), oxytetracycline (OTC), spiramycin (SPM), ampicillin (ABPC), colistin (CL), oxolinic acid (OA), and sulfadimethoxine (SDMX) were tested. Minimum inhibitory concentrations (MIC; μg/mL) were determined by the standard agar plate dilution method using the ZoBell 2216E medium. Each drug was diluted by the serial two fold dilutions from 100 to 0.025 μg/mL. Inoculated plates were cultured at 25°C for 24 hours.

Tissue NFS-Na Concentration after Bath Treatment

To determine the changes in tissue NFS-Na concentration, 15 tuna were treated with NFS-Na at 10 ppm for 30

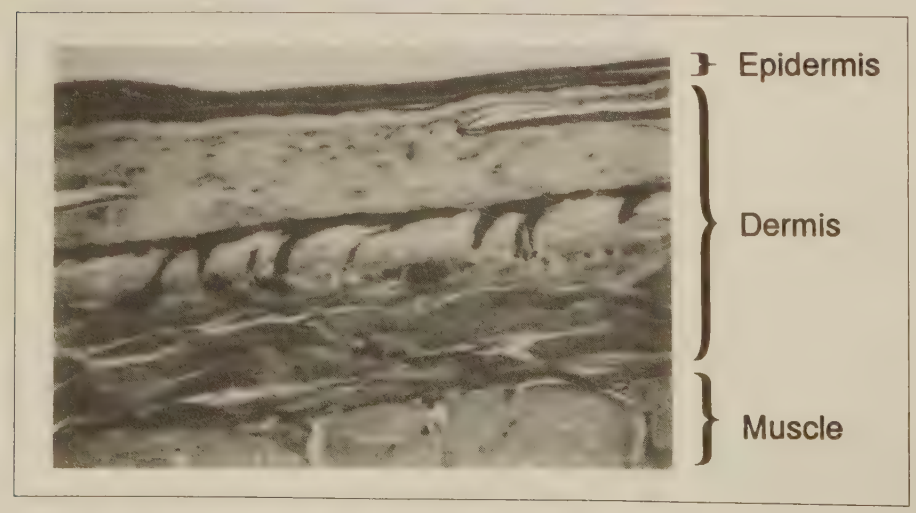

Figure 1
Structure of normal young bluefin tuna skin.

Figure 2
Ulcerated skin of young bluefin tuna.

minutes in a tank (8,000 L); two fish were sampled at 0, 2, 6, and 12 hours after treatment. The sampled fish were killed with a blow, blood was taken from the heart, and the liver, kidney, spleen, red muscle, and ordinary muscle were dissected and immediately frozen in acetone and dry ice. The plasma was separated by centrifugation and also frozen with dry ice. The frozen samples were later used for NFS-Na analysis. NFS-Na was extracted from homogenized tissues with ethyl acetate and purified using SEP PAK C18. Quantitative analysis of NFS-Na was performed with a high-performance liquid chromatography using Fine Gel 110 with eluent of acetonitrile-methanol-0.02 M phosphate buffer, pH 5.5 (2:5:3, v/v).

Results

Structure of the Skin

The skin of the young bluefin tuna is composed of two layers: the outer layer or epidermis, and the inner layer or dermis (Fig. 1). The epidermis consists of outer squamous epithelial cells, mucous cells, clavate cells, and germinal cells. The dermis consists of collagenous connective tissue layers of different densities. The inner margin of the dermis is bounded by a muscle layer abundant in blood vessels. There are layers of pigment cells in the marginal portions of the dermis, adjacent to both the epidermis and the muscle layer. Thus, the structure of young bluefin tuna skin is principally the same as that of other fish, although the skin of young bluefin tuna is very thin and fragile. Because of its fragile nature, handling results in the development of skin ulcers in young bluefin tuna.

Pathology of Skin Ulcers

The skin ulcer developed most frequently from the central to the posterior part of the lateral body. The ulcer was also commonly observed on the skin of the caudal fin, abdomen, head, and anterior part of the lower jaw (Fig. 2).

Exfoliation of the epidermis, hemorrhage in the germinal layer and both pigment cell layers, and infiltration of the lymphoid cells in the dermis were common pathologic features of the skin ulcer (Fig. 3). In severe cases lymphoid cells infiltrated the muscle layer causing the dermal layers to split. In more advanced cases the dermis completely exfoliated and the naked muscle was observed.

Bacteriological Examinations of Skin Ulcers

Severe skin ulcers appeared 24 hours after contact with the cotton glove. Development of the skin ulcer was fast probably due to the high water temperatures (25–30°C). All of the fish examined died within 48 hours after the glove contact. Changes in the bacterial flora and their numbers in the foci are shown in Table 1. The number of bacteria in the skin just after glove contact was $0.80–1.2 \times 10^3$

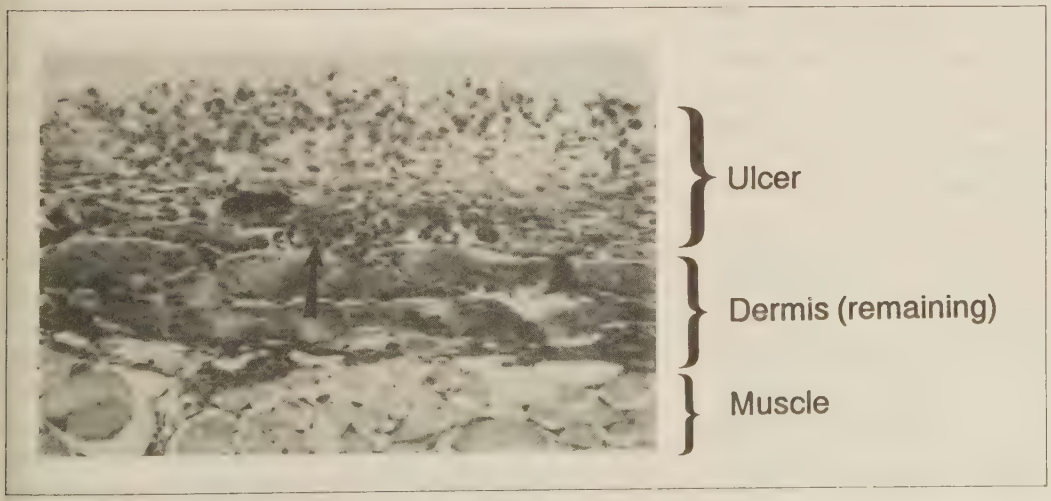

Figure 3
The foci of the skin ulcer of a young bluefin tuna. Epidermis is exfoliated and a large number of lymphoid cells have infiltrated the dermis. Disturbance of the dermal layer is also seen. Arrow indicates lymphoid cells.

Table 1
Numbers of bacteria and bacterial flora in the skin ulcers of young bluefin tuna after handling.

Time after handling (h)	Fish no.	Skin ulcer	No. of bacteria (cfu/cm^2)	Bacterial flora[a] (no. of colonies)								Bacteria isolated from kidney
				A	B	C	D	E	F	G	Total	
0	1	No	1.2×10^3	0	1	1	28	1	29	13	73	None
	2	No	8.0×10^2									None
	3	No	1.1×10^3									None
24	4	Severe	4.5×10^5	11	0	0	0	0	0	1	12	*Vibrio*
	5	Severe	2.6×10^6									*Vibrio*
	6	Severe	2.3×10^6									*Vibrio*
	Seawater		8.5×10^3/mL	11	3	0	1	0	0	10	25	

[a]Bacterial flora: A = *Vibrio*; B = *Pseudomonas*; C = *Moraxella* or *Acinetobacter*; D = *Flavobacterium* or *Cytophaga*; E = *Staphylococcus*; F = *Micrococcus*; and G = Others.

colony-forming units (cfu)/cm^2, increasing almost 10^3 times to $0.45–2.6 \times 10^6$ cfu/cm^2 within 24 hours. Just after handling with the glove, the genera *Flavobacterium/Cytophaga* and *Micrococcus* were dominant, and *Pseudomonas*, *Moraxella/Acinetobacter* and *Staphylococcus* observed in smaller numbers.* No bacterium was isolated from the kidneys of the fish at this stage. After 24 hours however, *Vibrio* became predominant in the more advanced ulcer foci and was also isolated from the kidneys of the fish at this stage. *Vibrio* was a predominant genus in the seawater used for the studies.

Chemotherapy of Skin Ulcers by NFS-Na

The survival of young bluefin tuna with or without NFS-Na treatment after transportation from the Kaminokae pens to Nansei are given in Figure 4. The number of deaths in the treated group was much smaller than that in the non-treated group for two days after the transportation. Almost all of the fish that died exhibited severe ulcers on their body surface. However, the ulcers of NFS-Na treated fish were generally less severe compared with those of nontreated fish.

Changes in the number of bacteria in the skin and kidneys after the bath treatment of NFS-Na are given in Table 2. Two hours after glove contact, the number of bacteria in the skin of treated and nontreated fish were nearly the same, and no bacterium was isolated from the kidneys of either group. After 24 hours the number of bacteria in the contacted skin was $0.4–1.4 \times 10^7$ cfu/cm^2 and $2.1–4.1 \times 10^7$ cfu/cm^2 for NFS-Na treated fish and

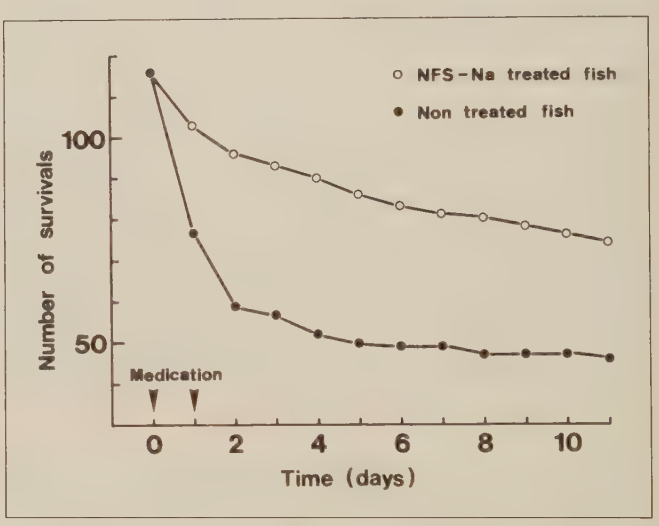

Figure 4
Effect of 5 ppm sodium nifurstyrenate bath administration for 30 min per day (2 days) on the mortality of young bluefin tuna after transportation from Kaminokae to Nansei by a fishing boat.

nontreated fish, respectively. At that time, $6.6–8.8 \times 10^4$ cfu/g kidney tissue of bacteria were isolated from the kidneys of nonmedicated fish, whereas no bacterium was isolated from those of NFS-Na treated fish. Bacteria were isolated from the kidneys of both treated and nontreated fish after 48 hours.

Changes in the bacterial flora in the skin and kidney after the bath treatment are given in Table 3. Before medication, *Pseudomonas*, *Moraxella/Acinetobacter*, *Flavobacterium/Cytophaga*, *Staphylococcus*, and *Micrococcus* were the main genera isolated from the skin. The native flora remained constant in NFS-Na treated fish after two hours, whereas *Vibrio* became predominant in the skin of nonmedicated fish. After 24 hours *Vibrio* was the predominant isolate

*Using only the several characteristics tested in this study, it was difficult to distinguish *Flavobacterium* from *Cytophaga* and *Moraxella* from *Acinetobacter*; therefore, we refer to them together.

Table 2

Effect of sodium nifurstyrenate (NFS-Na) bath administration on the number of bacteria in the skin ulcers and kidneys of young bluefin tuna.

Time after handling (h)	No. of bacteria (skin: cfu/cm^2; kidney: cfu/g)					
	NFS-Na treated fish			Nontreated fish		
	Fish no.	Skin	Kidney	Fish no.	Skin	Kidney
0				1	2.0×10^2	—[a]
				2	2.3×10^2	—
2	3	2.0×10^2	—	5	3.8×10^2	—
	4	5.0×10^2	—	6	4.0×10^2	—
24	7	1.4×10^7	—	9	2.1×10^7	8.8×10^4
	8	4.0×10^6	—	10	4.1×10^7	6.6×10^4
48	11	1.0×10^4	—	13	3.4×10^7	1.0×10^3
	12	1.4×10^6	1.9×10^4	14	1.0×10^4	—

[a] — = not detected ($< 1.0 \times 10^2$ cfu/g).

Table 3

Effect of sodium nifurstyrenate (NFS-Na) bath administration on bacterial flora in the skin ulcers and kidneys of young bluefin tuna. Bacterial flora: A = *Vibrio*; B = *Pseudomonas*; C = *Moraxella/Acinetobacter*; D = *Flavobacterium/Cytophaga*; E = *Staphylococcus*; F = *Micrococcus*; G = Others.

Time after handling (h)	Organ	Bacterial flora (no. of colonies)															
		NFS-Na treated fish								Nontreated fish							
		A	B	C	D	E	F	G	Total	A	B	C	D	E	F	G	Total
0	Skin	—[a]	—	—	—	—	—	—	—	0	6	8	3	1	4	2	24
2	Skin	0	4	3	15	0	0	2	24	11	0	1	1	2	0	5	20
24	Skin	0	23	0	1	0	0	1	25	23	1	0	0	0	0	0	24
	Kidney									24	0	0	0	0	0	0	24
48	Skin	10	4	0	0	0	0	2	16	15	0	0	0	0	0	0	15
	Kidney	16	0	0	0	0	0	0	16	1	0	0	0	0	0	0	1

[a] — = not tested.

found in both the skin and kidneys of nonmedicated fish, whereas *Pseudomonas* was most prevalent in the skin of NFS-Na treated fish. However, after 48 hours *Vibrio* also dominated the skin of treated fish and were isolated from the kidneys as well.

Sensitivities of bacteria isolated from the skin ulcers and kidneys to antimicrobial agents are given in Table 4.* The MIC of NFS-Na to the natural bacterial flora isolated from the skin before medication was 12.5–25 μg/mL for *Pseudomonas*, 25 μg/mL for *Moraxella/Acinetobacter*, 6.25–25 μg/mL for *Flavobacterium/Cytophaga*, 0.39 μg/mL for *Staphylococcus* and 3.13–6.25 μg/mL for *Micrococcus*. The MIC of NFS-Na to *Vibrio*, which was the primary pathogen isolated from the skin ulcers and kidneys of nonmedicated fish 2 and 24 hours after glove contact, was 0.10–0.78 μg/mL. The *Vibrio* spp. were also sensitive to CP, but less sensitive or resistant to OTC, SPM, ABPC, CL, OA, and SDMX. After 48 hours the MIC of NFS-Na to the *Vibrio* isolated from both the skin and kidneys of NFS-Na treated fish was 1.56–6.25 μg/mL.

Tissue NFS-Na Concentration after Bath Treatment

Tissue NFS-Na concentrations following a single bath treatment of 10 ppm of NFS-Na for 30 minutes are given

* Drug sensitivities were tested for several isolates of each genera. Because sensitivity was sometimes different among the isolates, we observed a range of MIC's within genera.

Table 4

Sensitivity of bacteria isolated from the skin ulcers and kidneys of young bluefin tuna to various antimicrobial agents. Abbreviations of antimicrobial agents: NFS-Na = Sodium nifurstyrenate; CP = Chloramphenicol; OTC = Oxytetracycline; SPM = Spiramycin; ABPC = Ampicillin; CL = Colistin; OA = Oxolinic acid; SDMX = Sulfadimethoxine.

Time after handling (h)	Medication	Site of isolation	Genus of isolates	No. of isolates tested	Minimum inhibitory concentration (μg/mL)							
					NFS-Na	CP	OTC	SPM	ABPC	CL	OA	SDMX
0	None	Skin	*Pseudomonas*	3	12.5–25	0.78–50	12.5–50	25–≥200	25–≥200	3.13	12.5–25	100–≥200
			M/A[a]	3	25	100	50	≥200	12.5	25	100	≥200
			F/C[b]	2	6.25–25	0.20–0.39	6.25–12.5	0.78–100	0.20–1.56	3.13	6.25–100	50
			Staphylococcus	1	0.39	1.56	≥200	0.78	0.05	—[c]	25	≥200
			Micrococcus	3	3.13–6.25	0.20–0.78	3.13–25	0.20–6.25	0.20–1.56	—	≥200	25
2	NFS-Na	Skin	*Pseudomonas*	3	12.5–25	0.78–1.56	25–≥200	100–≥200	0.78–6.25	3.13–≥200	3.13–6.25	12.5–100
			M/A	1	50	—	—	—	—	—	—	—
			F/C	2	6.25	—	—	—	—	—	—	—
	None	Skin	*Vibrio*	6	0.10–0.78	0.39–1.56	25–100	50–≥200	25–≥200	≥200	0.78–≥200	50–≥200
24	NFS-Na	Skin	*Pseudomonas*	6	3.13–50	0.10–0.78	25–50	25–≥200	0.78–3.13	1.56	3.13–≥200	12.5–≥200
	None	Skin, Kidney	*Vibrio*	6	0.20–0.39	1.56	50–100	≥200	≥200	≥200	12.5–≥200	≥200
48	NFS-Na	Skin, Kidney	*Vibrio*	7	1.56–6.25	1.56	100	≥200	≥200	≥200	≥200	≥200
			Pseudomonas	3	6.25–25	0.78–3.13	50–≥200	100–≥200	0.39–1.56	1.56–100	1.56–12.5	12.5–100
	None	Skin, Kidney	*Vibrio*	4	0.20–0.39	1.56	50	≥200	≥200	12.5–≥200	12.5–50	3.13–≥200

[a] M/A = *Moraxella/Acinetobacter*.
[b] F/C = *Flavobacterium/Cytophaga*.
[c] — = not tested.

Table 5

Changes in concentration of sodium nifurstyrenate in various organs of young bluefin tuna after bath administration of the agent. Assay sensitivity limit: 0.05 μg/g.

Time after medication	Fish no.	Concentration of sodium nifurstyrenate (μg/g)					
		Liver	Kidney	Spleen	Red muscle	Ordinary muscle	Plasma
0	1	0.07	0.42	0.08	0.41	<0.05	0.65
	2	0.10	0.51	0.16	0.31	<0.05	0.69
2	1	0.20	0.37	0.08	0.27	<0.05	0.36
	2	0.09	0.19	0.06	0.19	0.09	0.31
6	1	<0.05	0.11	<0.05	<0.05	<0.05	<0.05
	2	<0.05	0.24	<0.05	<0.05	<0.05	<0.05
12	1	<0.05	0.05	<0.05	<0.05	<0.05	<0.05

in Table 5. The NFS-Na levels in all tissues examined were highest just after the medication: 0.42–0.51 μg/g in the kidney, 0.31–0.41 μg/g in the red muscle, and 0.65–0.69 μg/g in the plasma. After 6 hours NFS-Na levels became undetectably low in all tissues except the kidney. NFS-Na levels in the kidney were similarly undetectable 12 hours after the treatment.

Discussion

The structure of the skin of young bluefin tuna is principally the same as that of other fish. However, the epidermal layer is very thin and fragile. It is evident that because of the fragile structure of the epidermis ulcers easily develop in the skin. The histopathological examination

revealed various kinds of inflammatory reactions and an increase of bacteria in the skin foci. The bacterial flora of the skin of healthy young bluefin tuna mainly consisted of *Pseudomonas*, *Moraxella/Acinetobacter*, *Flavobacterium/Cytophaga*, and *Micrococcus*. These bacteria are the common genera which appear on the skin of marine fish (Horsley 1977). *Vibrio*, which is a common bacterium in seawater, was not isolated from the skin of healthy young bluefin tuna. Thus, it seems that healthy young bluefin tuna have certain indigenous bacterial flora different from that of seawater. A facultative pathogen in seawater, *Vibrio* became predominant in advanced skin ulcers; the skin lesion it seems provides a favorable condition for *Vibrio* growth. The *Vibrio* sp. isolated from the kidney was identical with the ones which predominated in the foci of the skin ulcers; the skin ulcer is possibly an entrance for bacteria into the body.

Bath administration of NFS-Na to young bluefin tuna after the transportation from Kaminokae to Nansei decreased mortality probably by retarding ulcer development. As *Vibrio* isolated from the skin ulcer was highly susceptible to NFS-Na, it is clear that this chemical treatment suppressed the growth of the pathogens in the skin, slowing its invasion into the body, at least for a short period. Responses of various bacteria isolated from the skin to NFS-Na suggest that this medication inhibited the growth of *Vibrio* without influencing the indigenous bacterial flora in the skin. Thus, NFS-Na is considered an ideal antimicrobial agent to control skin ulcers. However, *Vibrio* resistant to NFS-Na increased in ulcer foci of the skin in advanced ulcers after 48 hours. In most cases a 10 ppm bath treatment increased tissue NFS-Na concentrations for several hours to higher levels than the MIC needed to inhibit *Vibrio* growth. However, the levels fell and were undetectably low within 12 hours. Therefore, it is necessary to treat with NFS-Na more than twice a day to maintain high tissue NFS-Na levels. Further studies are needed to determine the optimum schedule of NFS-Na treatment and to find more effective antimicrobial agents to control the skin ulcer of young bluefin tuna. *Vibrio* was also sensitive to CP. However, because CP is not legally permitted in fish culture in Japan, the use of this drug should be avoided.

Improvement of handling techniques is another way to reduce the occurrence of skin ulcers. We have found that a black polyethylene bag with many small holes is very useful for various kinds of handing. When a young bluefin tuna is introduced into the polyethylene bag in seawater, they usually become quiet in a short time. Although holding the fish with bare hands easily results in the development of skin ulcers, the handling of fish in the bag does not produce the severe ulcer.

Acknowledgments

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Successful HOTAC Methods for Developing Scallop Sowing Culture in the Nemuro District of East Hokkaido, Northern Japan

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ABSTRACT

Successful results with sowing culture (bottom culture) of the Japanese scallop, *Patinopecten yessoensis*, were achieved by using the "Hotate"-aid-conglomerate (HOTAC) methods in Nemuro district, which is located in east Hokkaido, northern Japan. Nemuro district scallop landings have increased more than tenfold in about ten years from the mid-1970s to mid/late-1980s. HOTAC methods were established using and integrated method (mandala) based on 1) the mass production of seed, 2) the control and monitoring of the entire culture system, and 3) the intellegent and proper use of human resources. The HOTAC operations contributed technological innovations in the areas of both hardware (e.g., larval monitoring devices, wide-area telemeter, HOTAC-modelled intermediate culture cage) and non-hardware (e.g., scientific knowledge, strategic manuals, use of human resources) components. Both components are being utilized on a widespread basis in Hokkaido. Consequently, the scallop landing in 1988 contributed a reliable 11.4% share to Hokkaido's fisheries production. The HOTAC achievement led clearly to a basic conclusion that the effective improvement of manpower founded on a science was the essential element among the non-hardware components which allowed the successful realization of an improved industrial scallop culture.

Brief View of the Japanese Scallop Fishery

In Japan, references to the scallop fishery usually refer to the Japanese scallop, *Patinopecten yessoensis*, fishery, which surpasses other scallop species in scale of landings. This fishery evolved rapidly after the 1970s because of the successful development of hanging (suspending) and sowing (bottom) culture in northern Japan, mainly in Hokkaido (Ito 1988; Figs. 1–2). Before the late 1960s, the scallop fishery depended on wild resources. Relatively high-level catches had been recorded until the mid 1940s (Tanaka 1963); however, low level catches occurred during nearly a quarter of a century period from the mid-1940s to the late-1960s. During the end of the 1960s, industrial scallop cultures developed owing to an innovation in seed production using wild spats (Ito 1986c; Ito et al. 1986). In 1988, the production of sowing culture and wild scallops amounted to 159,689 metric tons (t) and hanging culture reached 181,929 t, for a total production of 341,618 t. The value was 78,674 million yen (524 million U.S. dollars: calculated on the dollar = 150 yen).

The major landings have always been around Hokkaido in northern Japan. Scallops caught in Hokkaido represented 77% of all Japanese scallop production from both wild and cultured stocks during the period 1910–88. With the mid-1970s and the early-1980s an epoch ended, marking the beginning of the mass production period (Ito 1984a; 1984d; 1985a). During this period, serious problems with productivity stagnation were caused by mass mortality in hanging cultures, lack of seed for sowing culture, and industrial anxiety for a conventional culture method. So, a new culture technology was necessary to further develop the scallop culture industry; however, there was a lack of scientific knowledge to support the new epoch of a mass production period.

The author began to investigate the possibility of designing a new culture system for Japanese scallop in a typically

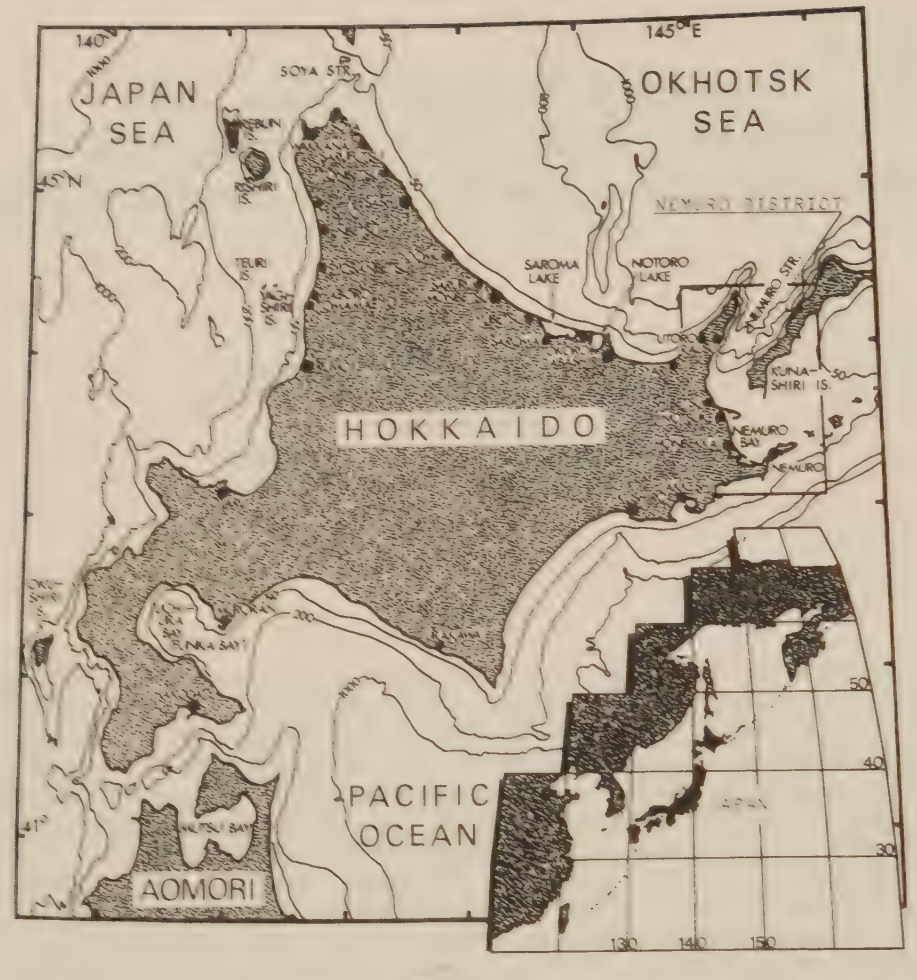

Figure 1
The Hokkaido and Nemuro Districts in Japan: contours are depths in meters.

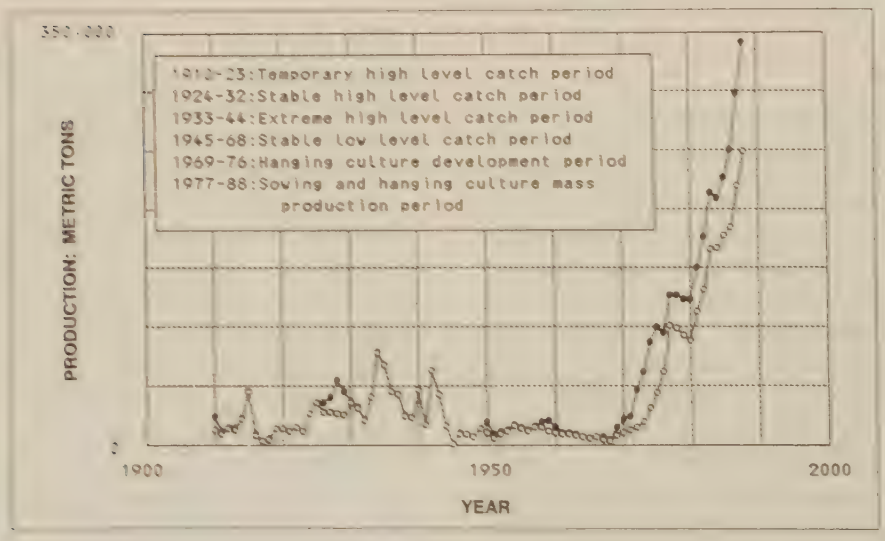

Figure 2
Yearly changes in fishery production, in metric tons, of the Japanese scallop, *Patinopecten yessoensis*, 1910–88. ● = total for Japan; ○ = total for Hokkaido. Source: Japan Ministry of Agriculture, Forestry, and Fisheries.

unproductive area such as Nemuro district in the mid-1970s (Ito 1983). At that time, efforts to culture scallops remained unsuccessful in Nemuro district because of many factors. This difficult state typified and emphasized the problems facing the Japanese scallop culture industry that occurred broadly over the northern Japanese scallop culture areas. This paper focuses on the establishment of an economical culture production for scallop fishermen using new and effective research methods.

Scallop Mariculture

The author has explained the concept (mandala) of Japanese scallop mariculture with a mandala diagram (Ito 1989a) because of the recent evolution of scallop culturing into a complicated industrial structure. The mandala concept comprehensively considers aspects of science, sociology, and economics. Technologically, the mariculture process of the Japanese scallop has three stages: a seed production phase, a culture phase, and a harvest phase (Fig. 3). The seed production phase is composed of two processes: wild spat collection and intermediate culture. The culture phase is performed by using one of two methods: sowing culture or hanging culture. During the harvest phase, sowing-cultured scallops are generally harvested by a scallop dredge when four years old, and hanging-cultured scallops are usually harvested at an age of two years old.

Background of Scallop Culture in Nemuro District

Before 1945, relatively high catches were recorded from Nemuro waters (Fig. 4), the value of which ranked second only to catches from the coastal waters of the Okhotsk Sea off Hokkaido (Tanaka 1963). This was caused partially by the higher level of wild stocks, which were sustainable owing to the relatively lower catch rates of the undeveloped or developing facilities (e.g., boats and gears) for scallop fishing. Secondly, Nemuro's fishermen utilized a wider fishing area, which before World War II included Kunashiri Island and the south Kuril Islands. After that time, the catch remained small until 1980 because of the low level of wild stocks (Fig. 5). The scallop fishery in Nemuro Bay was closed for two years (1975–76) owing to an exhaustion of resources (Ito 1983). Meanwhile, the establishment of the 200-nautical-mile exclusive zones reduced the Nemuro's fisheries production after 1977. Because the Nemuro community is based mainly on fisheries, its economy declined and required new resources. To solve this problem, an industrial program for scallop enhancement was started in the late 1970s, which employed sowing culture technique on a large scale. This start came to the Nemuro district later than other districts, such as

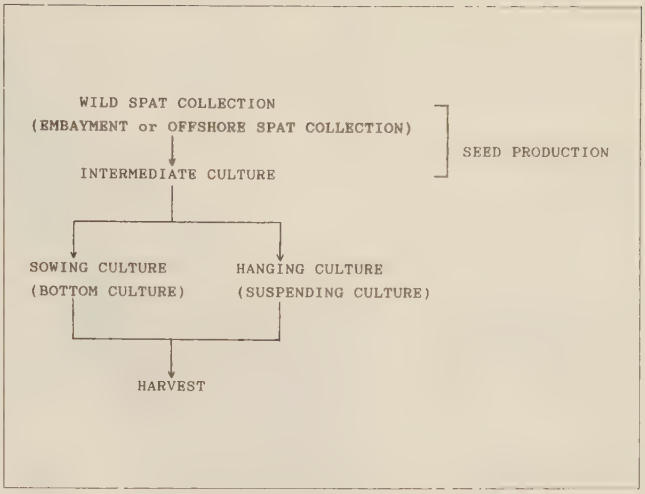

Figure 3
The process of Japanese scallop mariculture.

Abashiri and Soya on the Okhotsk Sea coasts, because there were obstacles to progress. In the meantime, a partial harvest had been realized from the extremely productive culture grounds off the coasts of Abashiri and Soya, although the culture technology remained undeveloped.

HOTAC Operations

The purposes of designing the industrial scallop culture program in Nemuro are summarized as follows: 1) to develop a sowing culture system, 2) to create commercial stocks, 3) to increase fishermen's incomes, and 4) to activate community economics (Fig. 6). However, there were four main barriers for the development of a culture system in Nemuro district (Ito et al. 1989):

- Limited seed supply:
  no spat because of unsuccessful wild spat collection,
  few seed supplied from seed market,
  mass mortality of hanging culture at seed supply areas.
- Limited amount of culture grounds:
  international dispute with the USSR,
  suitable bottom habitat in existing grounds limited.
- Starfish predation high in southern area.
- Weak manpower levels due to irregular flow and type of information on scallop culture technology provided to fishermen and persons concerned.

The essential roots of these problems were common to the general impediments in the Japanese scallop mariculture. The first was the lack of seed; no spats were available for seeding because of the unsuccessful efforts to

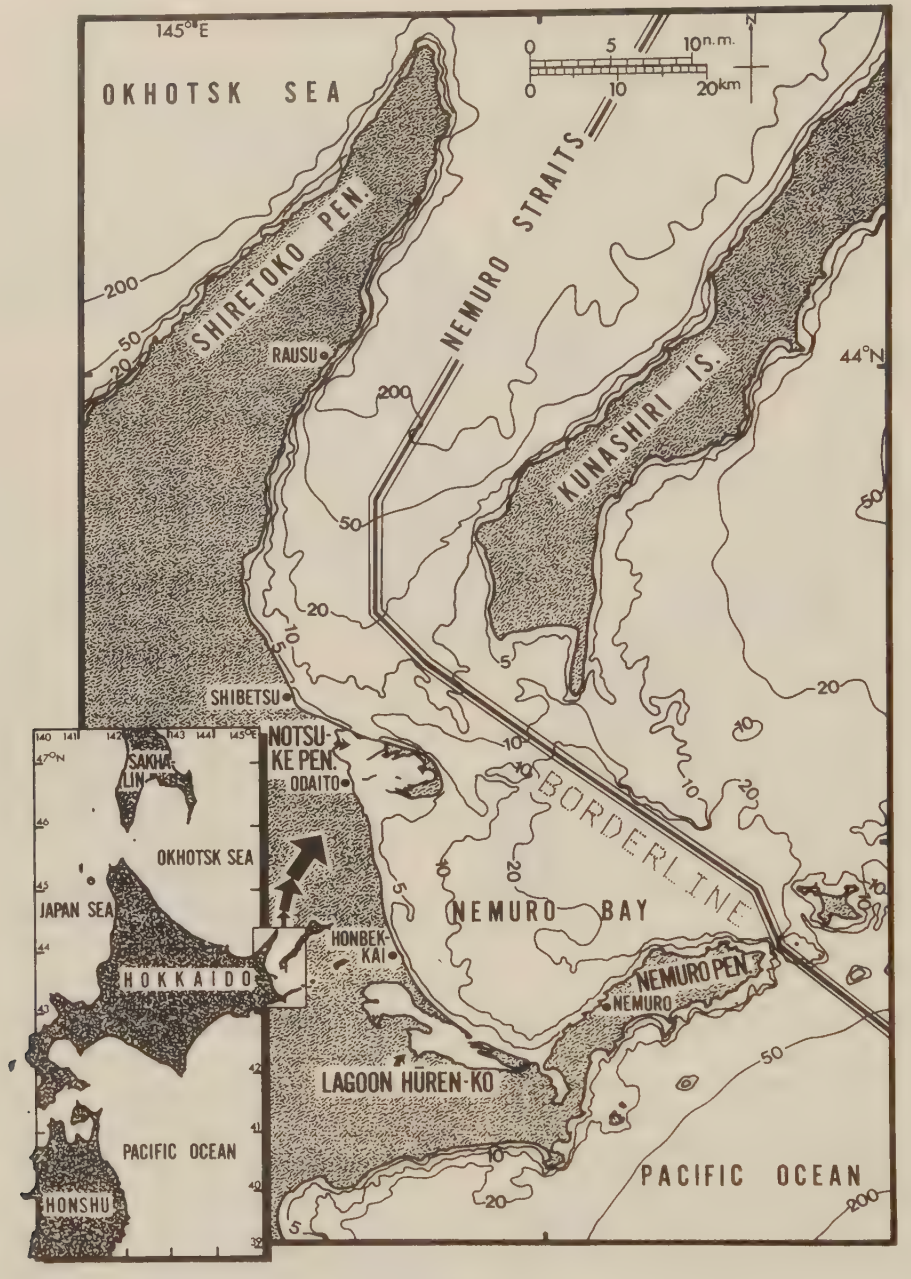

Figure 4
The Nemuro District in Japan: contours are depths in meters.

collect wild spat (Ito 1984c). Moreover, few seed were supplied from the main seed supplier areas in Funka Bay of southwest Hokkaido, which suffered mass mortality in their hanging cultures (Ito et al. 1986). Thus the seed production phase around Hokkaido suffered from both unsuccessful offshore wild spat collections and a chronically insufficient seed supply. The second problem was a limited area for culture grounds caused by both an international dispute with the U.S.S.R., which occupies territory eastward of Nemuro waters, and the limited amount of bottom habitat suitable as scallop habitat. As no culture ground is unlimited in size, the effective utilization of suitable areas is of general and current interest. The third was the large population of starfish in Nemuro Bay, south of Nemuro Straits. Only a few older scallops remained in the area (Ito et al. 1989; Fig. 7). Removal of a starfish population from a culture ground is an annual process for creating sowing culture, however, the starfish density in Nemuro Bay was the highest among Hokkaido coasts. The fourth problem was the lack of scallop enterprisers. A low morale existed because of irregular information available on current technology for scallop mariculture. In general,

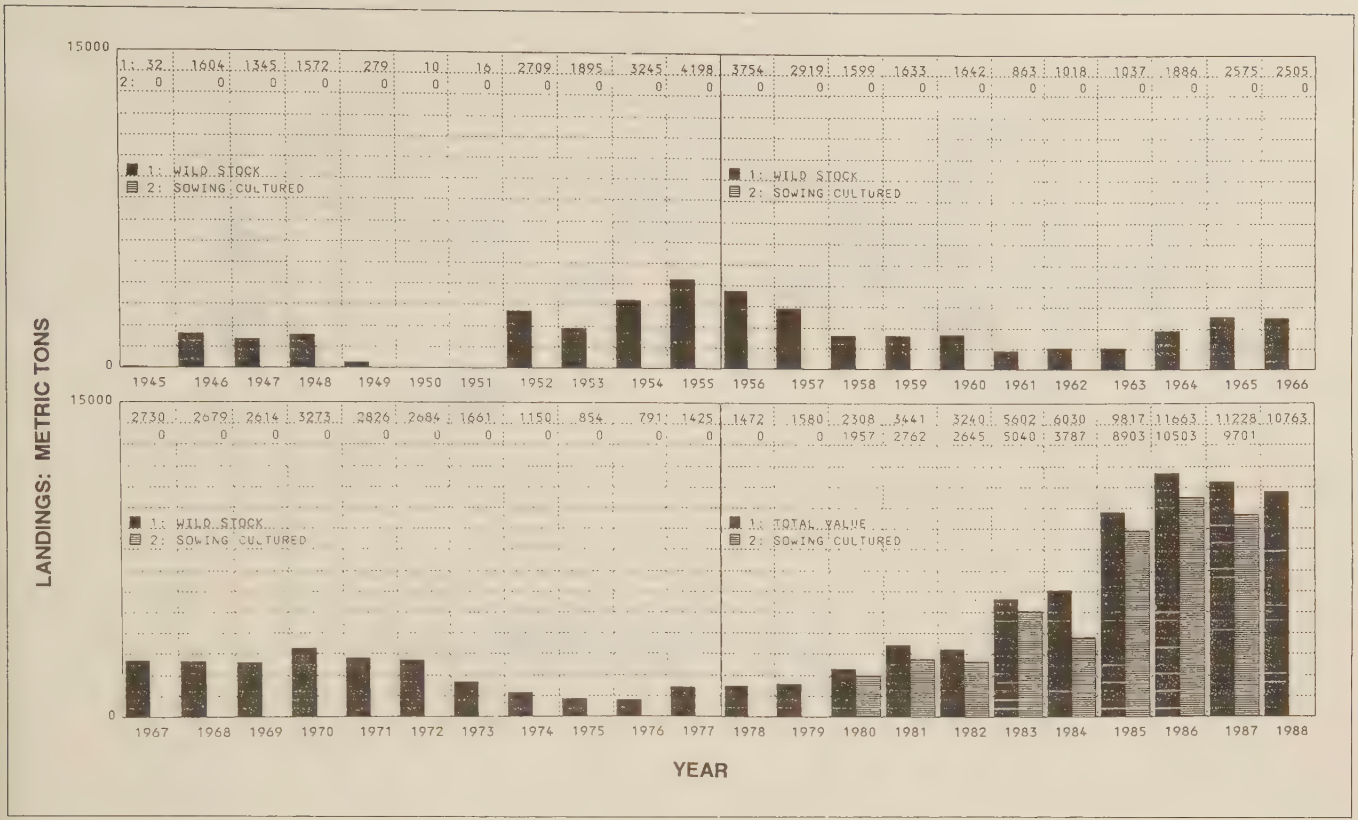

Figure 5

Yearly changes in the landings of Japanese scallop from Nemuro waters, 1945–88. Yearly totalled values are from Hokkaido Prefecture. The author compiled the values of the sowing culture harvest.

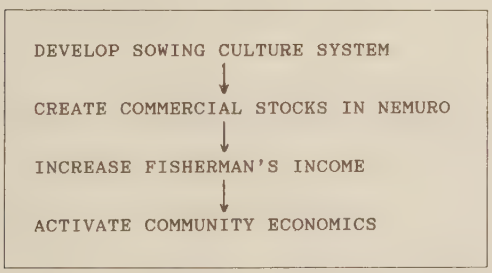

Figure 6

Purposes of the scallop culture program in Nemuro District.

many ignored the possibility that the use of manpower could be improved. At that time, the scallop culture technicians attempted to develop a primary stage; however, hasty conclusions were made. Many fishermen, culture technicians, administrators, and even onlooking scientists believed that the scallop mariculture could be easily established anywhere without further investigation. This was a serious problem; how to develop a scientific technology combining private enterprise and research investigations.

The author designed the further investigation with coworkers of acquaintances in the industry. We sought authorization for our large scale research that was to be combined with culture enterprise activities. There was little support and budget for the scientific research in this program by the official organizations which would contribute their activities to the improvement of the fishery. Therefore, the author formed a research team called the "Hotate"-aid-conglomerate (HOTAC), which consisted of coworkers belonging to several organizations concerned with scallop culture: Fisheries Cooperative Associations, Town Offices, Fisheries Extension Offices, and others. HOTAC was based on freedom of association, and its membership could be described as one based on "Jingi," a Japanese social code comprising justice, benevolence, and compassion. The HOTAC research was operated on a large scale. Although it required a large budget, there was no official support. We raised funding by gathering flexible and reasonable payment in our shares from the culture-related organizations. In time, a governmental research budget, within the limits of the Marine Ranching Program, eventually supported the program, but only after confirmation of successful harvests of the cultured scallop from Nemuro's grounds. The HOTAC research activities were

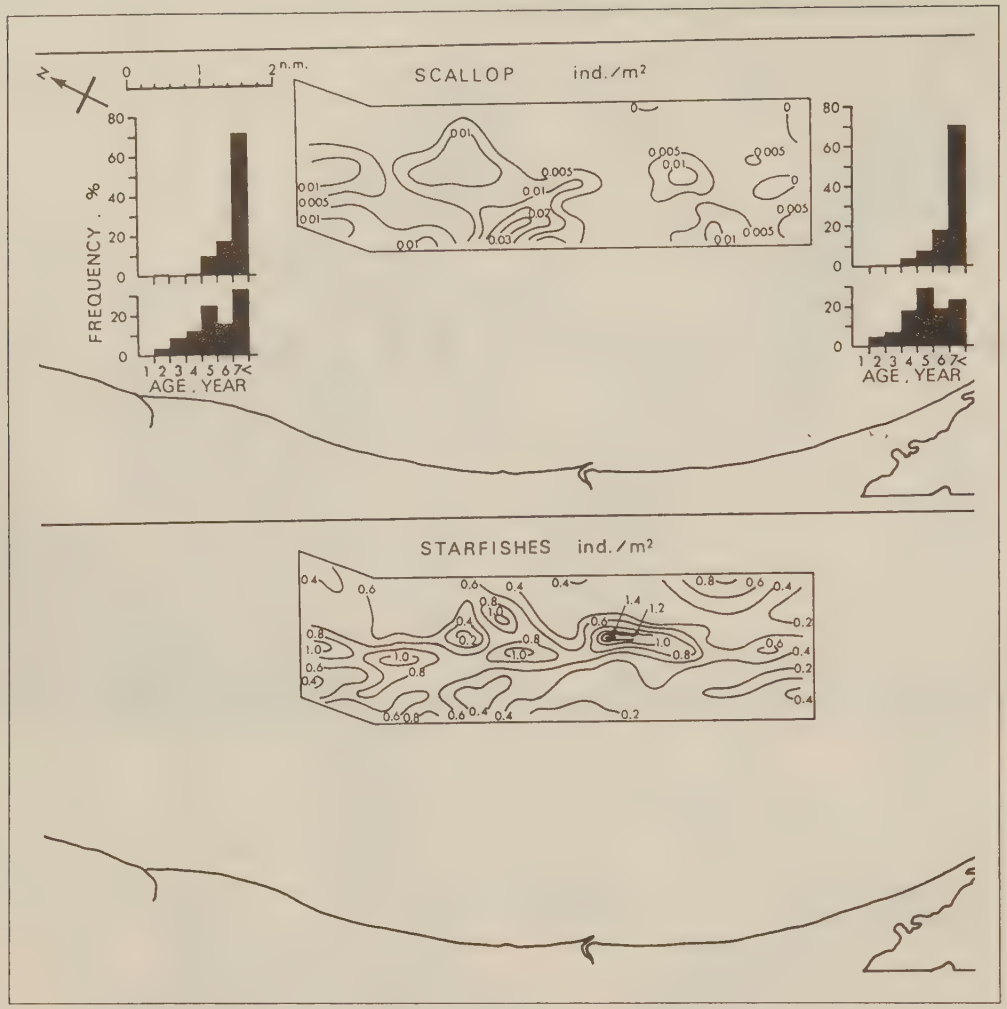

Figure 7

Distributions of the Japanese scallop and starfish in Nemuro Bay, south of the Nemuro Straights, during a period of low wild-stock concentrations of the scallop prior to the beginning of the sowing culture program in the late 1970s.

supported by their self-funded budgets during a critical duration of time. The HOTAC style of operating remains unique in Japan. HOTAC research methods were applied in three stages:

- Larval monitoring for wild spat collection by Fisheries Cooperative Associations and fishermen.
- Juvenile care check for intermediate culture.
- Population survey of cultured resources in a rotational planting ground.

A detailed HOTAC culture system was designed for Nemuro waters and its process is explained as follows:

- Seed production:
  wild spat collection,
  intermediate culture,
  if a lack of seed, introduction from seed market.
- Bottom habitat analysis.
- Removal of starfish.
- Seed sowing.
- Harvest.

First, there are the self-supplying seed production methods (seed collected by the fishermen themselves), which come from two new technological processes: one is for wild spat collection in waters less suitable for scallop culture where low densities of scallop larvae exist (Ito et al. 1988; Ito 1990; 1991, Fig. 8) and another is for intermediate culturing in waters that are frequently storm-tossed and visited by drift ice (Ito 1986b). Additionally, floating seeds from a seed market are introduced if self-supplied seeds are lacking. Usually, in Nemuro waters, there is a very low density of larvae and the opportunities to collect spats come too late. Conditions for wild spat

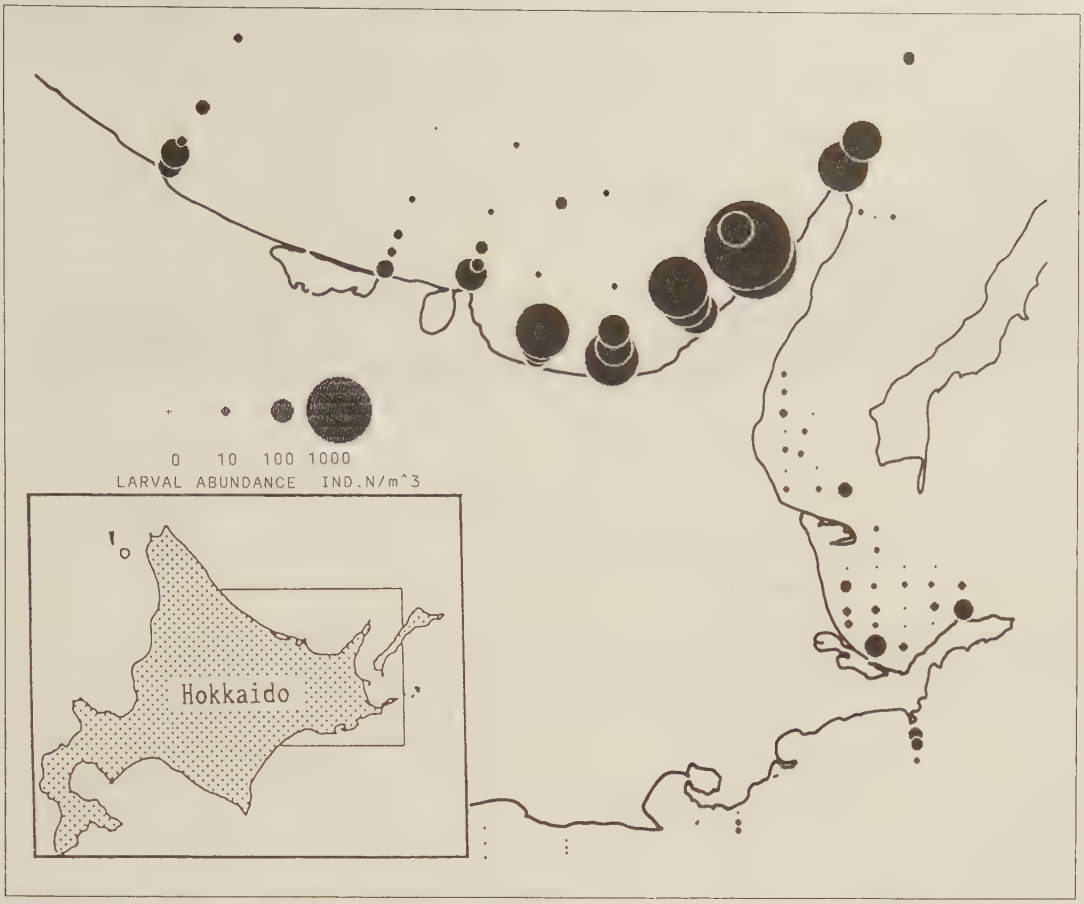

Figure 8
Typical larval distributions of Japanese scallop off east Hokkaido in early June. Although sufficient levels of larvae existed in the northern area, enabling spats to be collected, few larvae appeared in Nemuro waters in the southern area.

collection are not always critical in other waters off Hokkaido. Second, the bottom habitats are searched for the presence of cultures, and the particular culture grounds to be sowed are selected. Third, starfish are removed by scallop fishermen by means of scallop dredges and traps from the culture ground prior to seed sowing. One to three thousand metric tons of starfish were landed in a yearly clearance of every rotational planting ground in Nemuro Bay (Ito et al. 1989). In the fourth step, 50–150 million seeds are sown annually on selected rotational bottoms. Finally, the selectively sowed seeds are later harvested as four-year-old scallop from that particular culture ground.

An immediate feedback of the HOTAC operation results evolved making the new HOTAC methods innovative technology. HOTAC manuals, along with the following new hardware, were developed for the strategy designed from HOTAC operations:

- Computerized devices for scallop larval monitoring.
- Wide-area telemeter for culture condition monitoring.
- Effective HOTAC spat collector for lower larval density.
- Mass-productive HOTAC cage for intermediate culture.
- HOTAC anchor system of intermediate culture for clearing trouble of drift ice.
- HOTAC flow production system for mass seed production.
- HOTAC optional underwater TV system with high-fine camera for cultured population survey.

Fisheries Cooperative Associations and their fishermen only are permitted to conduct direct industrial actions related to fisheries (by the Japanese fishery law). Therefore, all industrial action of economic importance was accomplished by Fisheries Cooperative Associations and groups of scallop fishermen according to the HOTAC manuals under the control of the HOTAC culture strategy.

Results and Conclusions

After the HOTAC operations, commercial stocks were created from the sowing culture (Ito et al. 1987, 1988, 1989;

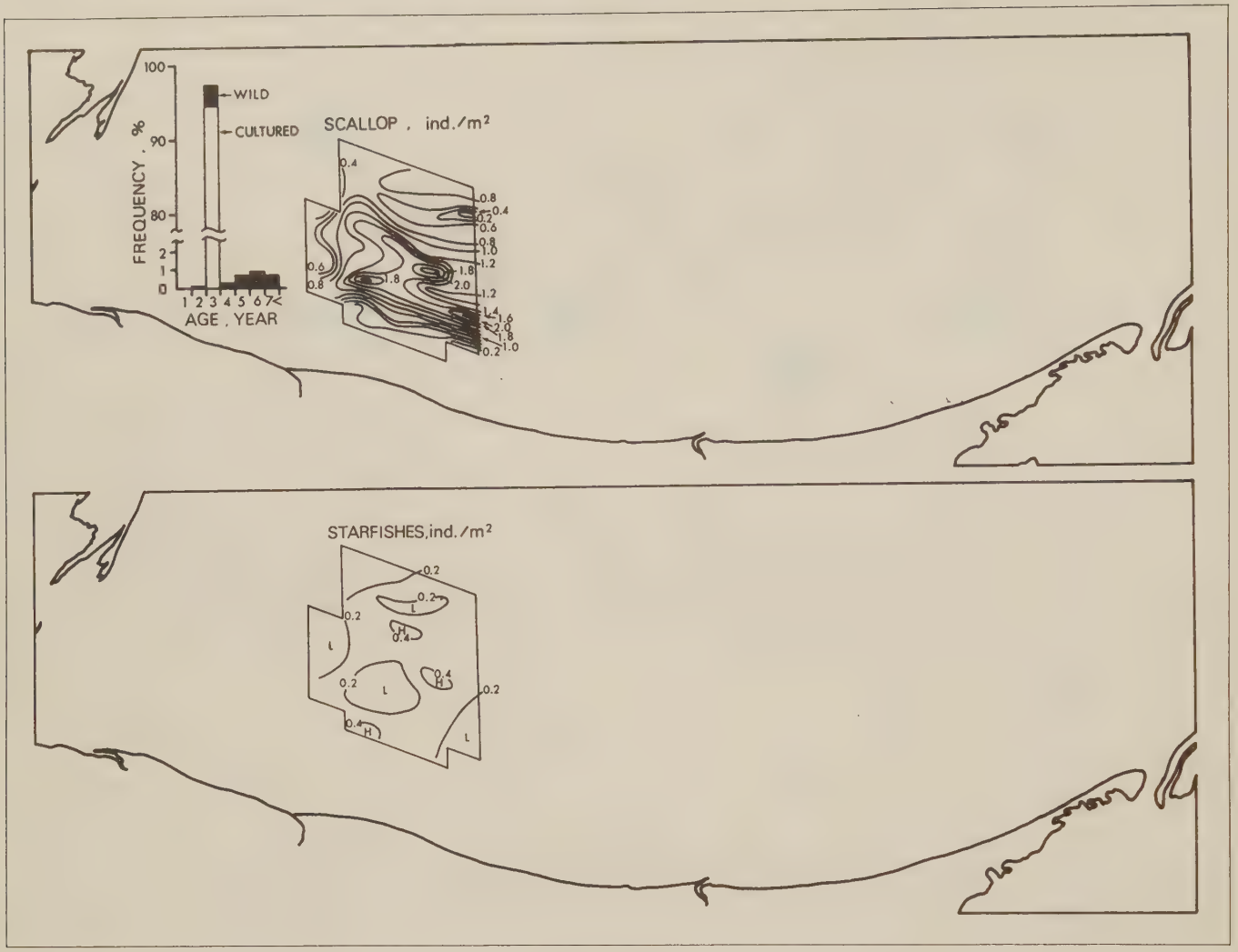

Figure 9

Distributions of sowing-cultured Japanese scallop and remaining starfish on a rotational culture ground in Nemuro Bay, south Nemuro Straits, after implementing culture operations (Ito et al. 1988).

Fig. 9) and fishermen harvested the cultured stocks and increased their incomes as follows:

- Commercial stock is created by sowing culture.
- Fishermen harvest improved resource and increase their incomes.
- Community economics become active.
- Culture system requires completion of essential elements: seed production, culture control, and creative use of manpower.

Moreover, the community became economically active, even though the fisheries activities in Nemuro district had previously fallen to a low level since the establishment of the 200-nautical-mile exclusive zones in 1977. In the mid-to-late 1980s, the scallop production value from Nemuro waters increased twelve times (eight times as adjusted by the consumer price index) from that of the mid 1970s (Fig. 10).

The HOTAC strategy of how to make a culture system formed from results of HOTAC operations, and from the following HOTAC-developed innovative technologies. Excellent wild spat collections from 1982 to 1988 were achieved with an explorative survey method for low density larvae (Ito 1990, 1991). The automated hardware for larval monitoring was developed with the support of special manufacturers (Ito 1985b, 1991). Marketable devices were obtained, such as the shape quantificator for discriminating species (CIA-HTT, Olympus Co. Ltd.) and the wide area telemeter for monitoring sea environments (SEACOM, Sanyo-suiro Co. Ltd.). A strategic manual is available for wild spat collection (Ito 1986a). Masses of qualified seeds were sowed on selected rotational bottoms (Ito 1989b). More productive seeds were produced with

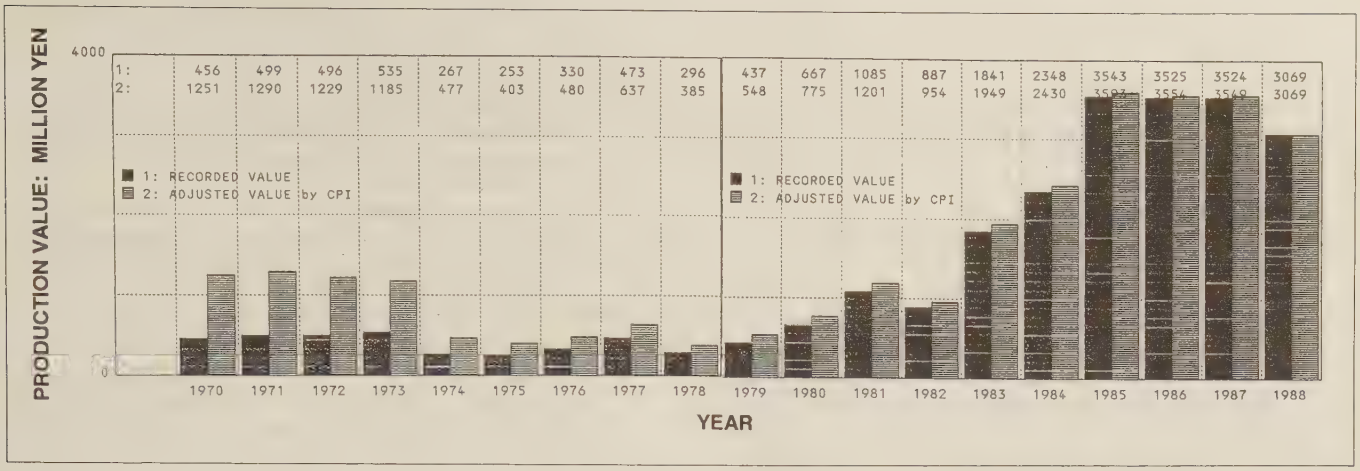

Figure 10
Yearly changes in the production value of Japanese scallop from Nemuro waters, 1970–88.

the novel mass production methods using new HOTAC-modelled cages (Ito 1984b; 1986b). In this industrial program, a successful method for wild spat collection and an effective culture system have been introduced and incorporated synchronously from the HOTAC operations by Fisheries Cooperative Associations and groups of scallop fishermen (Ito 1984c, 1984e). Some of the new hardware and non-hardware components of successful HOTAC results are being used on a widespread basis in Hokkaido. For example, spat collections in the open sea off Hokkaido have been stabilized by wide scale larval monitoring which directly imitates HOTAC methods (Ito 1991). This stabilization is due to the author and HOTAC members lecturing at every annual culturist meeting such as East and North Hokkaido Scallop Culture Technology Meeting since the early-1980s (Ito 1984a, 1986b).

In conclusion, the culture system requires the completion of three essential elements: mass production of productive seed, entire control of profitable culture, and intelligent and as well as proper use of human resources (Ito 1989a). The three elements are systematically related to each other in the mandala formation. The author explains with common Japanese terms that the three elements of seed production, culture control, and manpower improvement are "Tanezukuri," "Gyobazukuri," and "Hitozukuri," respectively. Basically, "Hitozukuri" or manpower improvement founded on science is the essential element other than equipment which allowed us to realize an industrial scallop culture.

Acknowledgments

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The Use of Aquaculture for Enhancement of the Common Property Fishery in Oregon, Washington, and Alaska

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ABSTRACT

Private and public sector aquaculture has been used for over 100 years in Pacific Northwest states to enhance wild stock salmon fisheries. The three states are compared regarding legal and regulatory constraints, operations, technical approaches, and measure of success. Various species are discussed along with problems associated with various culture regimes. Basic information on success or failure of programs is presented and species are cited.

Introduction

Oregon, Washington, and Alaska have developed different and unique approaches to enhancing the common property fishery in marine waters using aquaculture technology. This discussion, limited to salmonid species, describes the structure of each state program and its contribution to aquaculture methods.

State of Oregon

Some of the first private salmon hatcheries were constructed in Oregon in the 1870s. These private efforts were later replaced by public hatcheries in response to fishery declines and habitat loss from dam building on the Columbia River. In the late 1960s, there was resurging interest in salmonid culture in the private sector. In 1971, state legislation was passed, and a new private, commercial ocean-ranching industry created in Oregon. The original legislation authorized permits for chum salmon (*Oncorhynchus keta*) hatcheries only. In 1973, state law was amended to allow chinook (*O. tshawytscha*) and coho salmon (*O. kisutch*) permits and eventually (1979) ones for pink salmon (*O. gorbuscha*) as well (Ocean Resources Law Program 1979). The commercial salmon rancher must have a permit for each species of salmon it releases. The permits are issued by the Oregon Department of Fish and Wildlife after extensive review and public hearings. Various concerns are still being raised about commercial salmon ranching in the state. Potential genetic impacts and fish straying to or from private salmon hatcheries are the issues most often raised in this ongoing debate. Estimation of straying is not an exact science, and concern is expressed owing to the comparatively large number of salmon released by private hatcheries compared to releases from public hatcheries (Cummings 1987).

Oregon's private salmon ranching industry consists of twelve hatcheries with permits. The three largest operations release chinook, coho, and chum salmon: 1) Anadromous, with release sites at Coos Bay; 2) Oregon-Aqua Foods, with a release site at Newport; and 3) Oregon-Pacific Salmon Ranch, with a release site south of Gold Beach. The Domsea facility at Suislaw Bay last released fish in 1983. The eight other permits, issued for various locations along the Oregon coast, are for small operations allowed to release chum salmon only.

Oregon has experienced variable success in private salmon ranching over the last 15 years. The eight small growers have reared and released chum salmon with very little success (returns averaging less than one percent). The chum salmon have been found to have little or no resistance to bacterial disease in the hatchery and high mortalities have occurred. There are also unresolved questions relating to the carrying capacity of the estuarine systems where these fish have been released. Experimental results at Oregon State University have also been disappointing, where chum returns 1–1.5 percent of release. Chum salmon is not a major contributor to the private ocean ranching effort in Oregon and there is little evidence to indicate that

additional effort will occur in the near future (W. McNeil, Hatfield Marine Science Center, Newport, Oregon, pers. commun., Jan. 1989).

Most of the capital investment for ocean ranching has centered on chinook and coho salmon. It is estimated that more than $80 million of private investment has been expended for ocean ranching development in the past 15 years. The numbers of coho salmon released from private hatcheries gradually increased from 88,000 in 1974, to a peak 23.9 million in 1981 and 23.1 million in 1982 (Mayo 1988). Releases have declined since that peak effort. The three major companies plan to release over 5 million coho salmon in 1988. The average ocean catch plus the average return to private hatcheries for coho salmon from 1978 to 1987 was 2.24 percent while the combined average for 1985–87 was 4.55 percent (Mayo 1988). Improved return percentages were seen after the El Niño event of 1983–84. The contribution of private hatcheries to the commercial and recreational fishery (common property) has been significant in recent years. The average ocean catch for coho salmon between 1985 and 1987 was 662,000 fish. Sixteen percent (109,000) were from private ocean ranching hatcheries, nearly equal numbers (105,000) from the natural spawners, and the primary contribution (448,000) was from the state and federal hatchery systems (Mayo 1988).

Increased interest and effort has been placed on production of spring and fall chinook salmon since the early 1980s. Release efforts for chinook salmon have increased relative to the total numbers of coho salmon released, but the total chinook salmon release has been variable. Total releases of nearly 4 million chinook salmon occurred in 1983 and 1984 but declined to slightly over 2 million in 1986. The chinook salmon ocean catch plus returns to private hatcheries averaged 1.27 percent for 1978–87, but, like the coho salmon, was far better between 1985 and 1987, averaging 2.51 percent. In 1985, 35,000 chinook salmon were recaptured by private ranching facilities, and those numbers doubled for 1986 to 70,000 fish. The chinook salmon contribution to the common property fishery by private hatcheries in 1986 was 135,000 fish, but fell to 39,000 fish in 1987 (Mayo 1988).

Private ocean ranchers in Oregon have invested substantial capital in their operations during the last 15–20 years, but have yet to fulfill the early expectations. A great deal of technical information has been developed regarding management and husbandry, resulting in better knowledge and greater efficiency. The industry has gone through a number of managerial and proprietary changes which have slowed progress. In addition, regulatory changes by state agencies, state legislation (passed or proposed), and court decisions have complicated the development and expansion of ocean ranching in Oregon. The three major companies indicate that they are now marginally profitable or expect to be so in the near future. Each of the companies intends to proceed with their ocean ranching programs, but all are concerned about regulatory constraints that now exist or are being considered. Increased public understanding and awareness of the benefits of ocean ranching may be helpful to the industry in the future.

State of Alaska

Alaska first established a public salmon ranching program by an act of the state legislature in 1971. Recognizing the serious depletion of natural salmon runs statewide, the state took action and developed a comprehensive program. Three years later in 1974, the scope of the program was expanded with new statutes allowing the private nonprofit sector to participate in the rearing and release of salmonid species (Alaska Department of Fish and Game 1986). This combination of public and private nonprofit hatcheries has developed into the largest salmon ocean ranching program in North America. Sockeye salmon (*O. nerka*) production, as part of the total salmonid program, is the largest for that species in the world. The entire Alaskan program is approximately one-half the size of the Japanese ranching program and nearly equal to the Russian program. In 1987, the returns from both the public and private nonprofit programs accounted for about 20 percent of the total salmon harvest statewide (Allee 1988).

Presently, the ocean ranching program is administered by the Alaska Department of Fish and Game, Fisheries Rehabilitation, Enhancement and Development (FRED) Division. In 1988, the FRED Division operated 16 hatcheries plus pathology, limnology, and coded-wire tag laboratories. Three hatcheries previously operated by FRED are now being operated by the private sector through a contractural agreement. Other FRED programs include lake stocking, lake fertilization, comprehensive salmon planning, private nonprofit hatchery permitting, and stream rehabilitation projects.

Administration of the various public hatcheries is divided into the following five regions: Southeast, Prince William Sound, Cook Inlet, Kodiak and Alaska Peninsula, and the Arctic-Yukon-Kuskokwim. Data and statistics are kept for production, release and return for each species in each region. In general, the primary emphasis is on pink and sockeye salmon, followed by chum, coho, and chinook salmon. In addition, rainbow trout and steelhead (*O. mykiss*), grayling (*Thymallus articus*), and char (*Salvelinus* sp.) are also managed. Each area has specific management plans directed at specific enhancement goals for that region as part of the state plan. Releases of fish from FRED administered facilities increased from 388.4 million fish in 1987 to 412.7 million in 1988. The estimated fishery contribution by FRED hatcheries and projects in 1988 was 5,847,076 salmonid fishes (Holland 1989). Of that total, the commercial catch represented 3,589,545, the sport

catch equaled 841,222, and 1,416,309 fish were accounted for as brood stock.

Some in Alaska described 1988 as the year of the enhancement-produced fish. Extremely low natural returns would have resulted in poor harvests in many areas, had it not been for the hatchery programs. In 1988, 91 percent of the entire lower Cook Inlet pink salmon catch and 79 percent of the sockeye salmon catch came from fishery enhancement projects. The Prince William Sound region experienced a similar situation with 90 percent of the pink salmon caught originating from the hatchery programs. Planted sockeye and chum salmon also contributed to the Prince William fishery. In essence, economic disaster in the commercial fishing industry in some regions of Alaska was averted in 1988 by the significant contribution of state and private sector hatcheries.

The private nonprofit hatchery (PNP) program was created in 1974 and established under the administration of the Alaska Department of Fish and Game. The PNP program, administered by the FRED division in cooperation with the department's fisheries management divisions, carries out regulatory responsibilities related to public and private aquaculture in Alaska. Seven regional PNP associations have also been formed which cooperate with the Alaska Department of Fish and Game in developing and maintaining salmon production plans and rehabilitation and enhancement activities. Each association comprises a representative from the commercial, sport and subsistence fishermen, as well as members of local communities. Regional planning teams develop regional salmon plans which are mandated by regional charter. The funding necessary for the PNP corporations has been obtained primarily through a revolving loan fund administered by the Alaska Department of Commerce and Economic Development. Loans up to $10 million are available for individual projects with a pay-back period of over 30 years (Alaska Department of Fish and Game 1986). A total of 29 PNP hatchery permits have been issued since 1974; three permits have been given up. Twenty-two of the hatcheries with permits are in operation and 19 had returns of adult salmon in 1988. In addition, 36 scientific/educational permits for aquaculture research projects or school district programs were issued in 1988 (Holland 1989).

It was estimated that 14.3 million adult salmon, originally released as juveniles from PNP facilities, were harvested in common property fisheries or returned to hatchery harvest areas in 1988. In Prince William Sound, PNP hatcheries contributed an estimated 8.7 million pink salmon, representing over 87 percent of the total pink salmon harvest in that area (Holland 1989).

Clearly the FRED Division and PNP hatcheries have made significant and substantial contributions to the common property fishery in Alaska through their public and private aquaculture programs. Unfortunately, reductions in state funding for the FRED Division have occurred each year since 1986. A key issue in the debate over funding is whether primary users should pay an increased share of the enhancement costs. Fishermen assert that they are large contributors to the state's general fund and that additional fees should not be imposed on them for enhancement. This political controversy has been debated since 1986 and will most likely continue to be an issue in the Alaskan ocean ranching program.

Another controversy regarding aquaculture also exists. Development of salmon net-pen farming in the private sector has created a situation that has resulted in a two year moratorium. Commercial fishermen concerned with potential biological and economic problems have successfully lobbied the state legislature to stop or stall the development of this new industry. Net-pen farming advocates point to state documents that indicate the net economic gains from net-pen culture and cite positive examples of salmon farming in Europe and Canada as reasons to create a favorable climate for similar developments in the state. The biological potential appears to be unlimited, but the political controversy is intense.

State of Washington

Private, for-profit ocean ranching is not presently allowed in the state of Washington. In the late 1970s and early 1980s this issue was debated in the state legislature and bills were introduced; however, efforts to establish private salmonid enhancement were rejected. Numerous issues emerged during the debate, a few of which are worth exploration.

By the 1960s and 1970s, the Pacific Northwest had come close to losing major runs of Columbia River salmon. Some of the Columbia runs were even considered for endangered species status. At one time, annual anadromous salmonid runs were as high as 15 million fish per year, but numerous impacts had drastically reduced those former numbers. Losses from hydro-power dams were cited as the primary cause, but reductions also occurred from irrigation, flood control, overfishing, and poor logging, grazing, and farming practices. Similar concerns existed for the Puget Sound runs because of habitat loss and overfishing. In 1977, the state established an objective of increasing the public catch of salmon by Washington citizens and appropriated $30 million for this effort. Again, in 1980, an additional $70 million was authorized for the Salmon and Steelhead Conservation and Enhancement Act (Washington State Department of Fisheries 1988). To some, it appeared that private ocean ranching would be consistent with these efforts, but numerous questions and political disputes emerged.

During the debate, the Washington Department of Fisheries (WDF) remained neutral but provided important information related to commercial ocean ranching. The

three areas sited for special attention were 1) biological considerations; 2) regulatory considerations; and 3) fiscal considerations. There was a dearth of information regarding the biological questions. What would result if large numbers of fish were released into finite systems which had undetermined carrying capacities? Examples were cited where enhancement of some species may have impacted other species within the region. Numerous variables existed, but ultimately there was no clear demonstration that private ocean ranching would result in a net gain to the public fishery. The regulatory questions (area 2) related to the priority of ocean ranching versus enhancement of the existing salmon resource as administered by the department. The fiscal concerns (area 3) questioned the ability of WDF to administer a new ocean ranching program under the existing budget. As previously stated, the option for commercial ocean ranching was not approved by the Washington State Legislature.

The existing enhancement programs into open waters of the state include both public and private efforts. The public effort consists of the Washington Departments of Fisheries (anadromous fish) and Wildlife (steelhead), the University of Washington School of Fisheries, and the U.S. Fish and Wildlife Service. Private non-profit organizations (sport fishing and enhancement groups) release fish through cooperative efforts with WDF. Indian tribes release fish as part of their commercial effort and therefore, are the single exception to the prohibition on private for-profit ocean ranching in the state. The tribes, as sovereign nations, are not constrained by state laws; however, close cooperation exists between state, federal, and tribal hatcheries that assures coordination of effort for the various enhancement programs.

The Washington Department of Fisheries is the lead agency and the primary source of salmonid enhancement within the state. For the five years from 1983 to 1987, the WDF released an average of 246 million salmon each year. The total release effort from all sources was 341 million fish in 1986 and 337 million fish in 1987. These yearly releases are critical to the maintenance of the salmonid runs in the waters of the state. A summary of total plantings by all contributors in 1987 is listed below:

WDF hatcheries — 249.6 million
WDF cooperatives — 12.2 million
Tribal hatcheries — 42.0 million
U.S. Fish/Wildlife — 33.7 million
University of Washington — 0.2 million
(Abrahanson 1988).

Survival rates of released fish are quite variable depending on species and time and point of release. In an effort to increase survival rates, many fish are now impounded in freshwater and marine net pens. Free release into the environment has been delayed for a few weeks to a few months allowing the fish to acclimate and grow in confined protection. In early testing in South Puget Sound, coho salmon survival, using traditional release technology equaled 10 percent, while the use of delayed-release sites increased survival to 14.5 percent. Estimates in other areas indicate even higher survival rates depending on the species and the management strategies used. Presently there are 25 saltwater delayed-release facilities operating in Puget Sound. Delayed-release facilities also operate on the coast and in the Columbia River.

An impressive example of the net pen delayed-release programs in South Puget Sound is the program operated jointly by WDF and the Squaxin Tribe, located at Squaxin Island. The program provides a substantial fishery for the tribe, as well as other commercial and sports fisheries in the area. There was virtually no commercial coho salmon fishery in the Squaxin Island area prior to the project in the early 1970s. In an early study (1974–79), the tagging survey revealed an annual recovery of 17.1 percent (Rensel et al. 1988). The commercial net fishery benefited most from the program, and as a result of that early success, the delayed-release program in that area has made a tenfold increase in their release effort.

Net-pen sites also exist as part of the volunteer enhancement program. An important part of this effort was stimulated with the passage of the Washington State Volunteer Cooperative Fish and Wildlife Act of 1984. This is a popular and successful program. In 1988, WDF issued approximately 150 permits for cooperative projects. These efforts include net pens, egg tubes and egg boxes in streams, aquariums in schools and small hatcheries constructed and operated by volunteers. There have been recent proposals to increase these efforts, especially net-pen facilities. There is even interest by some commercial fishermen to establish private, nonprofit programs modeled after the Alaskan programs.

A small, but efficient farmed salmon industry also exists in Puget Sound. The industry has grown slowly over the past 20 years, and 13 farms are now established and growing fish. Production is primarily Atlantic salmon (*Salmo salar*), but coho and chinook salmon and steelhead are also being grown. Fish farmers have offered to provide unused portions of their net pens as part of the enhancement effort in the state. If utilized, this effort would be in cooperation with the WDF. Although enhancement could be supplemented in this way, the future expansion of the salmon farming industry is presently uncertain. Demographic and use conflict constraints have resulted in polarization of the issue by affected parties. Resolution of the problem may ultimately occur in the court system, unless some acceptable compromise can be developed.

In 1986, salmon represented 25 percent of the total harvest (202.5 million pounds) of all marine shellfish and finfish caught or cultured in Washington State (Hoines et al. 1987). The estimated contribution from release of

cultured salmonids for common property enhancement varies by region and species. At least 65 percent of the total Puget Sound fishery harvest is provided by enhancement programs. The estimate for the coastal sport and commercial fisheries, which are dependent on the Columbia River system is even higher, at 80–85 percent. The stated goal of WDF is to perpetuate and enhance the runs of salmon by operating a hatchery system to produce artifically reared fish and by enhancing spawning runs with the protection and improvement of salmon habitat. It is clear that a viable and sustainable long term salmon fishery could not exist in Washington State without enhancement programs. The future of enhancement will depend on continued state and federal support and the continued cooperation of volunteerism.

Conclusion

Creative solutions to maintain our salmon resource for the future must be supported and constantly re-evaluated. Aquaculture, both private and public, has played an important role in the history of enhancement of the salmonid common property in the waters of Alaska, Washington, and Oregon. Without development of the necessary management and husbandry technologies, viable runs of salmon would have been lost. It remains to be seen what role the private and public sectors will play in salmonid enhancement. The uncertainties of federal and state funding, participation and contribution by the commercial industry, the role of tribes, and the expansion of private farming and ranching will all affect enhancement in the future.

As the demand for all fishery products for consumption and industrial use continues to expand at a rapid rate, the limited resource becomes more valuable. The record market values for salmon in 1988, which parallel most fishery products, is an example of the market demand driving the price of the product. Numerous fisheries around the world have been depleted and the world catch remains static while demand outstrips supply. Aquaculture, both private and public, will play an ever-increasing role in solving this problem, and should be viewed as a tool to be used wisely for the benefit of present and future generations.

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Incidence of Fish Pathogenic Viruses among Anadromous Salmonids in the Northern Part of Japan, 1976–1987

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ABSTRACT

During the period from September 1976 to December 1987, various species of mature salmonid fish, including masu (*Oncorhynchus masou*), chum (*O. keta*), pink (*O. gorbuscha*), kokanee salmon (*O. nerka*), charr (*Salvelinus leucomaenis*), and rainbow trout (*O. mykiss*), were examined to provide information on the distribution of pathogenic viruses in northern Japan. Virus inspections were conducted on ovarian fluids, mixed kidney and spleen specimens, epithelial tumor tissues, and blood samples. Four viruses were isolated during the course of this investigation. Infectious hematopoietic necrosis virus (IHNV) was found in the ovarian fluid of chum and masu salmon. *Oncorhynchus masou* virus (OMV), discovered in 1978 and specific to masu salmon, has been isolated from ovarian fluids and epithelial tumor tissues at 13 sampling sites. Chum salmon virus (CSV) was isolated from mixed kidney and spleen specimens from healthy chum salmon in 1978 and again in the ovarian fluids of masu salmon in 1987 at two localities on the coast of the Sea of Japan. Infectious pancreatic necrosis virus (IPNV) was isolated from masu salmon at two locations: once from tumor tissue in 1981 and a second time from an ovarian fluid sample in 1987. Viral erythrocytic necrosis (VEN) was found at four locations in the erythrocytes of chum and pink salmon in waters along the Okhotsk coast. Cytoplasmic particles with a hexagonal profile were found in the erythrocytes by electron microscopy.

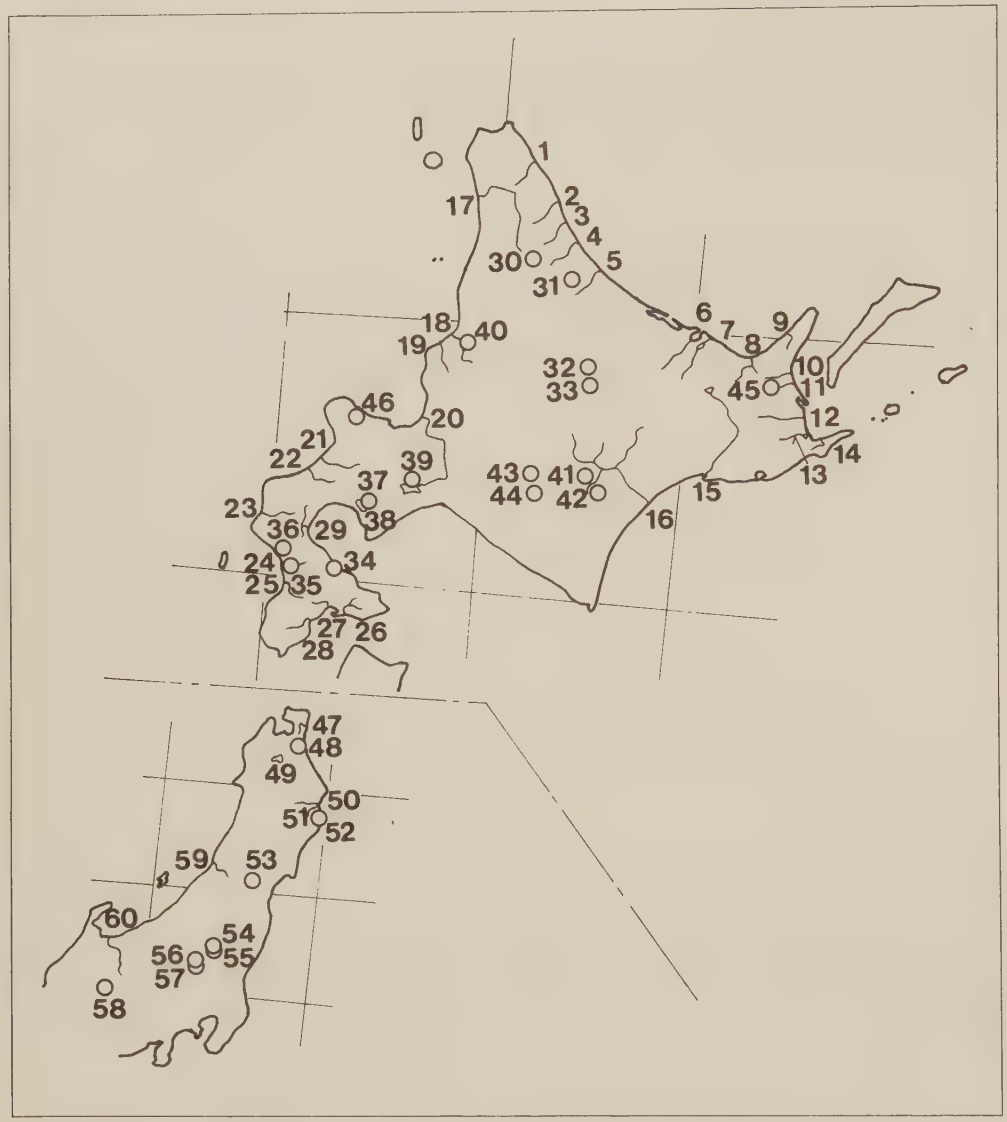
Introduction

Information on the distribution and incidence of fish pathogenic viruses is important for the prevention of transmission to the progeny of mature salmonids. Therefore, we studied the occurrence of pathogenic viruses among mature salmonids in the northern part of Japan. Here, we introduce the results of our investigation from September 1976 to December 1987.

Materials and Methods

Fish Used

From September 1976 to December 1987, we collected 6125 ovarian fluid specimens from 6 species of 11,095 females and 21 seminal specimens from 2 species of 155 males of mature salmonid fishes. Until 1978, 100 fish were sampled at each collection site and were pooled into 10 specimen lots. Subsequently 60 fish were used, and



1: Tonbetsu R.
2: Kitamihorobetsu R.
3: Tokushibetsu R.
4: Horonai R.
5: Okkoppe R.
6: Tokoro R.
7: Abashiri R.
8: Shari R.
9: Iwaobetsu R.
10: Ichani R.
11: Shibetsu R.
12: Nishibetsu R.
13: Fuuren R.
14: Bettoga R.
15: Kushiro R.
16: Tokachi R.
17: Teshio R.
18: Nobusha R.
19: Shokanbetsu R.
20: Chitose R.
21: Shiribetsu R.
22: Shubuto R.
23: Toshibetsu R.
24: Toppu R.
25: Assabu R.
26: Shiodomari R.
27: Hekirichi R.
28: Shiriuchi R.
29: Yuurappu R.
30: Bifuka
31: Nishiokkoppe
32: Kamikawa-A
33: Kamikawa-B
34: Mori H.
35: Otobe H.
36: Kumaishi H.
37: Toya L.H.
38: Toya L.
39: Shikotsu L.H.
40: Nobusha H.
41: Memuro
42: Sarabutsu
43: Hidaka-A.
44: Hidaka-B.
45: Nakashibetsu
46: Shakotan
47: Oippe R.
48: Aomori P.F.E.S.
49: Towada L.H.
50: Hei R.
51: Tsugaruishi R.
52: Tsugaruishi H.
53: Yamagata P.F.E.S.
54: Chuuzenji L.H.
55: Nikko H.
56: Gunma-K P.F.E.S.
57: Gunma-H P.F.E.S.
58: Gifu P.F.E.S.
59: Miomote R.
60: Jintsu R.

Figure 1

Waters in Hokkaido and northern part of Honshu where salmonid populations were sampled and examined for presence of IHNV, OMV, IPNV, and CSV, and coastal waters of Okhotsk where certain species were examined for VEN, 1976–1978. O = Hatchery or fish farm; R = River; H = Hatchery; L = Lake; P.F.E.S. = Prefectural Fisheries Experimental Station.

specimens were collected individually. Species sampled included masu (*Oncorhynchus masou*), chum (*O. keta*), pink (*O. gorbuscha*), and kokanee salmon (*O. nerka*), charr (*Salvelinus leucomaenis*), and rainbow trout (*O. mykiss*) from the following 60 collecting stations: catching stations (29 in Hokkaido, 5 in Honshu), hatcheries (6 in Hokkaido, 9 in Honshu), 10 fish farms and 1 lake all in Hokkaido. From 1981 to 1987, 140 tumor tissues observed among 4115 fish were used for *Oncorhynchus masou* virus (OMV) inspection. Furthermore, 190 mixed kidney and spleen specimens were taken from 858 of these fish and blood smears prepared from 660 fish were employed for virus inspection and for microscopical examination for evidence of viral erythrocytic necrosis VEN, respectively. Thin sections of blood of these fish were observed by electron microscopy (EM). Collection sites of the specimens are noted on the map of Figure 1.

Collection of Ovarian Fluid Specimens

Ovarian fluid specimens were collected according to the method of Yoshimizu et al. (1985). A sterilized automated pipette tip was inserted into the urogenital opening of the mature fish. One mL of ovarian fluid was taken from the fish and sterilized by one of two methods. Until 1981, a filtration method with a millipore filter HA (0.45 μm) was employed and subsequently the antibiotic treatment method of Amos (1985). Both filtrate and antibiotic treated specimens were transported to the laboratory in ice.

Virus Inspection and Identification

RTG-2 (Wolf and Quimby 1962) and CHSE-214 (Fryer et al. 1965) cell lines cultured in roller tubes or 24-well tissue culture plates were employed for virus inspection. We inoculated 0.1 mL of specimen into 2 tubes or wells, and observed them for 10 days at 15°C. Isolated viruses were identified using the rabbit antisera against infectious hematopoietic necrosis virus (IHNV), OMV, and infectious pancreatic necrosis virus (IPNV), and chum salmon virus (CSV). Some ovarian fluid specimens that showed positive results of virus inspection were measured for virus titer using the RTG-2 cell line with the microtiter plate. For the inspection of VEN, smears of erythrocytes were fixed by methanol, stained by 10% Giemsa solution, and viewed by light microscopy (× 400). Thin sections of the erythrocytes were prepared from specimens in which we found inclusion bodies, and the virus particles were observed by E.M.

Isolation of the Virus

From 1981, epithelial tumor tissues observed around the mouth were used for the OMV inspection according to the method of Yoshimizu et al. (1987). Tumor tissue was cut off from the fish and disinfected with iodophore (50 ppm, 15 min), washed with Hanks' balanced salt solution (HBSS) containing antibiotic, and brought to the laboratory with ice.

Light and Electron Microscopy

Prior to egg collection in female fish, blood was collected from the veins under the backbone of the tail for electron microscopy; 1 or 2 drops of blood was fixed in 1 mL of 1.25% glutaraldehyde with 0.05M phosphate buffer's saline (PBS, pH 7.2) and 4% sucrose. After 1-h fixation, the blood was washed with PBS, centrifuged at 3000 rpm for 10 min, and postfixed in 2% osmic acid. After collection of the eggs, blood was collected in a capillary tube from a small hole opened in the kidney and spread on a glass slide. After air-drying, it was fixed with methanol for 20 min and stained with 10% Giemsa solution.

Results and Discussion

IHNV

Results of the virus inspections are shown in Figure 2. Also included are the results of the examination of blood smears for VEN. IHNV was isolated from the ovarian fluid (each of 10 pooled specimen lots) of 100 chum salmon at the Abashiri River in 1976 and at the Yuurappu River in 1977. In the following year, IHNV was discovered at the Mori Hatchery in masu salmon; the incidence of infection was 60 percent. For three consecutive years, from 1979 to 1981, the entire physical facilities at Mori received an annual disinfection with chlorine, while the eggs were treated with iodophore. As a result of this cleaning project, IHNV has not been isolated again from mature masu salmon at the Mori Hatchery until now (Awakura, unpubl. data).

In 1980, IHNV was isolated from rainbow trout at the Aomori Prefectural Fisheries Experimental Station and from kokanee salmon at the Towada Lake Hatchery. The incidence of infection was 8 and 3 percent, respectively, increasing the next year to 42 and 70 percent. In 1982, infection rates at the Towada Lake Hatchery had increased 98% owing to a failure to disinfect the facilities (Yoshimizu et al. 1988a). The difference between Mori and Towada lake Hatcheries suggests that to prevent IHNV outbreak, early measures to disinfect the eggs and facilities are very important. IHNV was also isolated from rainbow trout at the Chuzenji Lake Hatchery in 1983 with a frequency of 8 percent.

Recently, in 1985, IHNV was isolated from the ovarian fluid of masu salmon taken from the Shari River; the incidence of infection was 60 percent. In this case, infectivity of IHNV in the ovarian fluid was measured at 10^2 $TCID_{50}$/mL with the exception of 2 fish whose infectivity was 10^4 $TCID_{50}$/mL. All eggs and facilities had been disinfected by iodophore before the early eyed stage, thus avoiding an outbreak of IHNV.

In Hokkaido, most of the hatcheries culturing masu salmon also culture chum salmon. when we compared the susceptibility of chum and masu salmon to IHNV, chum salmon showed low mortality (less than 25%), compared with masu salmon (Yoshimizu et al. 1989). Recently, an epizootics of IHNV among chum salmon at Kitoi, Russell Creek, and Eklutna in Alaska was reported (Follett 1987) and again at Iwate Prefecture in Japan (Yoshimizu et al. 1988b). Thus IHNV is not a virus to be neglected when raising chum salmon.

OMV

OMV was first isolated from ovarian fluid specimens of masu salmon at the Otobe Hatchery in 1978 with an infection rate of 7.5 percent. In 1978 the rate increased to 61 and in 1983 to 75 percent (Yoshimizu et al. 1988a).

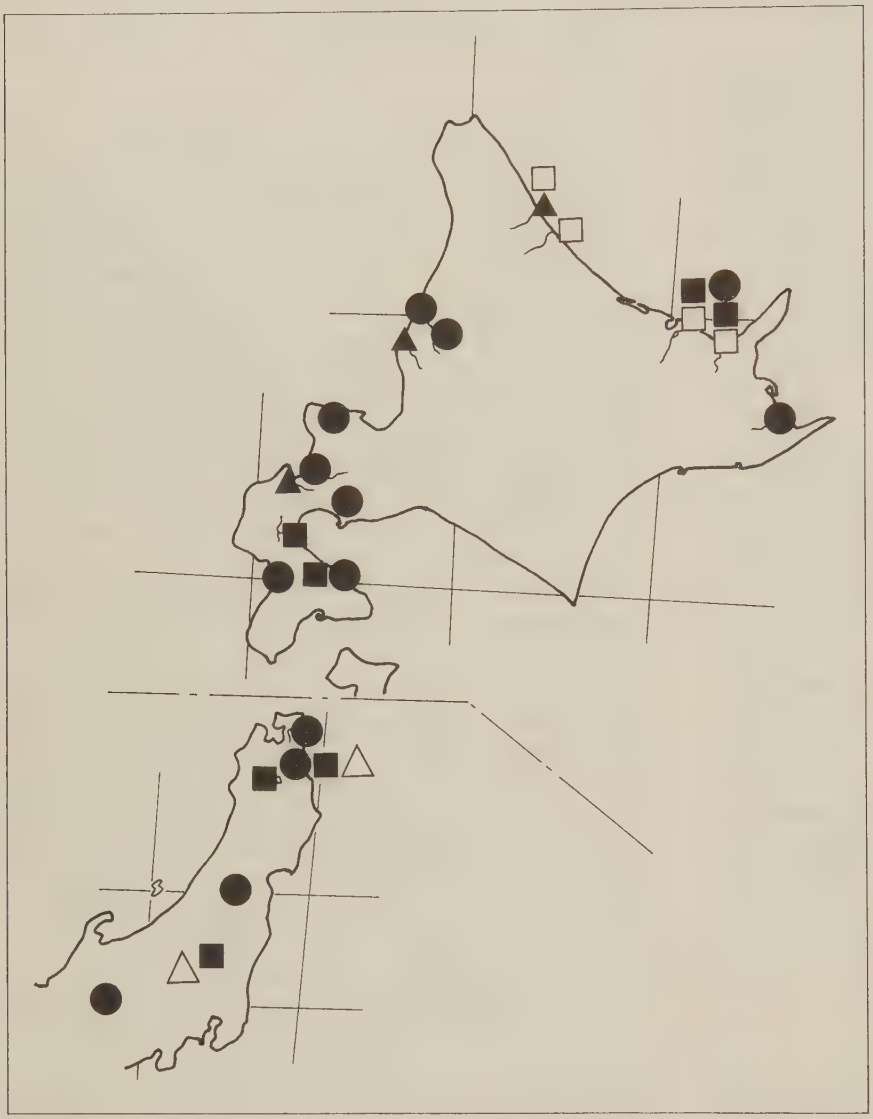


Figure 2
Location of rivers, lakes, hatcheries and fish farms where IHNV, OMV, IPNV, and CSV were isolated; and coastal waters where VEN infected fish was found, 1976–1987. ■: IHNV; ●: OMV; △: IPNV; ▲: CSV; □: VEN.

Over an 8-year period the virus would be detected in fish at a total of 13 locations in northern Japan. This virus was specific to masu salmon and could not be isolated from other species of salmonid fish. Recently, a herpes virus neutralized with anti-OMV rabbit serum was isolated from juvenile coho salmon (*O. kisutch*) cultured in fresh water (Horiuchi et al. 1989). *Renibacterium salmoninarum* and *Flexibacter columnaris* were also isolated at the same time.

It appears that OMV belongs to the herpes viruses and has a pathogenicity to salmonid fish, as well as an oncogenicity (Kimura et al. 1980, a and b; 1981, a, b, and c; 1983; Yoshimizu et al. 1987). Although the size of this virus is 200 to 240 nm (Kimura et al. 1981a), a 0.45 μm membrane filter reduced the infectivity by 99.4 percent. In 1982 we decided to compare the filter sterilization and antibiotic treatment methods using ovarian fluid sampled at the Otobe Hatchery. Although OMV was not isolated from the filter sterilized specimens, it was found in 21 percent of the samples treated with antibiotic only. Because of these results, we changed the method of sterilization of ovarian fluid specimens to the antibiotic method (Yoshimizu et al. 1988a). Following that change, we isolated OMV from six locations that year: the Otobe, Mori, and Shakotan Hatcheries, the Aomori P.F.E.S., and the Shiribetsu and Oippe Rivers (Yoshimizu et al. 1988a). The following year (1983), OMV was discovered at the Fuuren River to an even greater degree. From the autumn of 1983, when we suggested iodophore treatment at the early eyed state, the number of places where OMV has been isolated has decreased and in 1986, OMV was not isolated from the localities where we collected specimens (Yoshimizu et al. 1988a).

From 1981, we observed the body surface, especially around the mouth, and found epithelial tumors at 12

localities. The same basal epithelial tumors have been previously reported at several localities in Japan (Kimura 1976). Sano et al. (1983) also isolated the herpes virus, yamame tumor virus (YAV), from tumor tissues of masu salmon at Niigata Prefecture. OMV was isolated from all the epithelial tumor tissues used for the virus inspection by means of either the primary culture of co-culturing method (Yoshimizu et al. 1987). Tumors induced by OMV were histopathologically similar to those of the tumors observed on the parent fish (Yoshimizu et al. 1987).

At the Otobe Hatchery, OMV was isolated annually from 1978, and the incidence of tumor bearing fish was increasing. In 1983, we examined the infectivity of various organs of masu salmon at the hatchery. Although OMV could be isolated from the ovarian fluid specimens of these fish, OMV could not be isolated from the kidney, spleen, liver, intestine, and heart tissues. The infectivity of the OMV in the ovarian fluid was also low, ranging from $10^{1.8}$ to $10^{2.1}$ $TCID_{50}$/mL. In the autumn of 1984, all fish cultured in this facility were killed and the facility disinfected with chlorine. Then fish were transplanted from Kumaishi Hatchery where no virus had been isolated. Because Otobe Hatchery did not keep brood stock, we could not check the mature fish; however, tumors induced by OMV were not recognized among the fingerlings (Yoshimizu et al. 1988a).

In the case of the Aomori P.F.E.S. in 1981, three kinds of viruses, OMV, IHNV, and IPNV were isolated from the same tumor tissue. Additionally, in the case of the Shiribetsu River (1983) all the fish bearing the tumor were tagged, indicating they had been cultured and released from the Shiribetsu Hatchery (Yoshimizu et al. 1988a). OMV was isolated from either ovarian fluid or tumor tissue at all 13 locations where we examined more than 60 individual specimens (except 4 hatcheries). This suggests that OMV is distributed widely in the northern part of Japan.

CSV

CSV (chum salmon virus) (Winton et al. 1981) was isolated from kidney and spleen mixed specimens collected at Tokushibetsu River in 1978 (Winton et al. 1981). This virus was recognized as an orphan virus and did not show severe pathogenicity for salmonid fish (Winton et al. 1981). In 1986 an unknown disease broke out among masu salmon near Tokushibetsu, and CSV was isolated from the diseased fish (Yoshimizu 1988). The next year, in 1987, CSV was isolated from ovarian fluid of mature masu salmon at Shokanbetsu River and Shubuto River, both located on the Sea of Japan coast. We need to study the pathogenicity of CSV in masu salmon.

VEN

An agent of viral erythrocytic necrosis (VEN) could not be isolated with the tissue culture method. We therefore used Giemsa stain for erythrocytes and observed inclusion bodies. In 1980, 1 out of 60 chum salmon collected from the Abashiri River showed a positive result and, in 1981, the same inclusion bodies were found in chum and pink salmon collected from the Tokushibetsu River, Horonai River, and Shari River, and again in the Abashiri River. We found the iridovirus in thin sections of erythrocytes of chum salmon collected from the Abashiri River (Yoshimizu et al. 1988a).

IPNV

IPNV was isolated from tumor tissue of masu salmon with OMV and IHNV at Aomori P.F.E.S. in 1981 and also from the ovarian fluids of masu salmon cultured at the Gunma P.F.E.S. in 1987 (Yoshimizu et al. 1988a). According to the annual reports of the Hokkaido Fish Hatchery (1976, 1981, 1982), IPNV has been isolated from the ovarian fluid of masu salmon, rainbow trout, and coho salmon cultured in fresh water fish farms, but the prevalences were not high.

Conclusion

From the results of this investigation, IHNV, OMV, IPNV, CSV, and the agent of VEN were distributed widely in the northern part of Japan. In most cases, fish infected with these viruses were masu salmon. An effective method for reducing the incidence of these pathogenic viruses is presently needed.

Four viruses and an agent of viral erythrocytic necrosis were isolated during the course of this investigation.

1. IHNV was isolated at 7 collection sites from either masu or chum salmon.

2. OMV was first discovered in masu salmon in 1978. OMV was isolated from ovarian fluid or epithelial tumor tissues. The incidence of OMV was decreased when we suggested iodophore treatment at the early eyed stage.

3. CSV was discovered in healthy chum salmon at the Tokushibetsu Hatchery in 1978 and again from the ovarian fluids of masu salmon at two places on the Sea of Japan coast in 1987.

4. IPNV was isolated from tumor tissue of masu salmon at Somori P.F.E.S. in 1981 and also from ovarian fluid of masu salmon at Gunma Prefecture in 1987.

5. VEN was found in the erythrocytes of both chum and pink salmon taken in the waters along the Okhotsk coast.

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Ecology and Production of Fish in a Man-made *Sargassum* Forest

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ABSTRACT

Investigations were carried out to clarify the effects of a man-made *Sargassum* forest on fish production, which is needed to support the planned enlargement of the coastal fisheries resource. The main fish species gathering in the forest included *Sebastes inermis*, *Sebastiscus marmoratus*, and *Hexagrammos agrammus* which appeared year round, and *Halichoeres poecilepterus* and *Navodon modestus* which appeared seasonally. In spring the maximum forest size is reached as well as the number of phytal animals (e.g., Caprellidea, Gammaridea, Mollusca, and Polychaeta). The latter are suggested to be the important food resource of these fish.

Introduction

It has been pointed out that natural *Sargassum* forests play an important role in fish production by their impact on the ecology of fish (Fuse 1962). Recently, artificial *Sargassum* forest formations have been examined to increase fishery resources by expanding the habitable area for fish and shellfish. Various animals gather and form a new community in the man-made forests thus enlarging animal production. There are various relationships between different fish and the forest. Some fish species gather to eat phytal animals (e.g., caprellids, gammarids, molluscs, and polychaetes) and others to hide. Investigations were carried out to quantitatively analyze these situations and clarify the effects of man-made *Sargassum* forests on fish production (Okamoto et al. 1987, 1988).

Materials and Methods

The man-made *Sargassum* forests are located off the coast of Ihota, Yashiro Island, Yamaguchi Prefecture, in an area 170 × 50 m at a depth of 3–6 m. There are about 700 concrete blocks (mainly 0.75 × 0.53 × 0.53 m) placed on sand which are divided into 30 groups of different size (16–50 blocks) (Yoshikawa and Tsukidate 1987, 1988). The species composition, size frequency, and behavior of fish gathering there was determined by net catches and underwater observations which were carried out once to three times a month from 1986 to 1987. Four kinds of trinal trammel net with different center-net mesh sizes (25.0, 18.8, 15.0, 10.7 mm) were combined (with lengths 60, 60, 60, 30 m, respectively, all heights 1.5 m) and set from the evening to the next morning for 16–18 hours, which corresponds to unit effort. Similar catches were done by surrounding one group of *Sargassum* forest (4 × 4 m) composed of 16 blocks (spaced 0.5 m apart from each other) with a trinal trammel net of 15.0 mm mesh size. After measuring the captured fish, stomach contents were fixed and preserved in 10% formalin, and analyzed later. Phytal animals were collected by diving with 0.3 mm mesh nylon nets, then fixed and preserved in 10% formalin. Later they were completely separated from seaweed contaminants by being filtered through a 0.5 mm mesh sieve (Okamoto et al. 1987, 1988).

Results and Discussion

Fifty fish species were found from December 1986 to November 1987. Twenty of these were rare species. The appearance rate of the most common 24 species is shown in Figure 1 by the CPUE (catch [in number] per unit effort) along with the density of the man-made *Sargassum* forest at the time of collection (Yoshikawa and Tsukidate 1987, 1988). There were four types of fish as follows: A type, appearing almost year-round, including *Ditrema temmincki*, *Neoditrema ransonneti*, *Rudarius ercodes*, *Sebastes inermis*, *S. hubbsi*, *Sebastiscus marmoratus*, *Hypodytes rubripinnis*, *Hexagrammos otakii*, *H. agrammus*, *Pseudoblennius cottoides*;

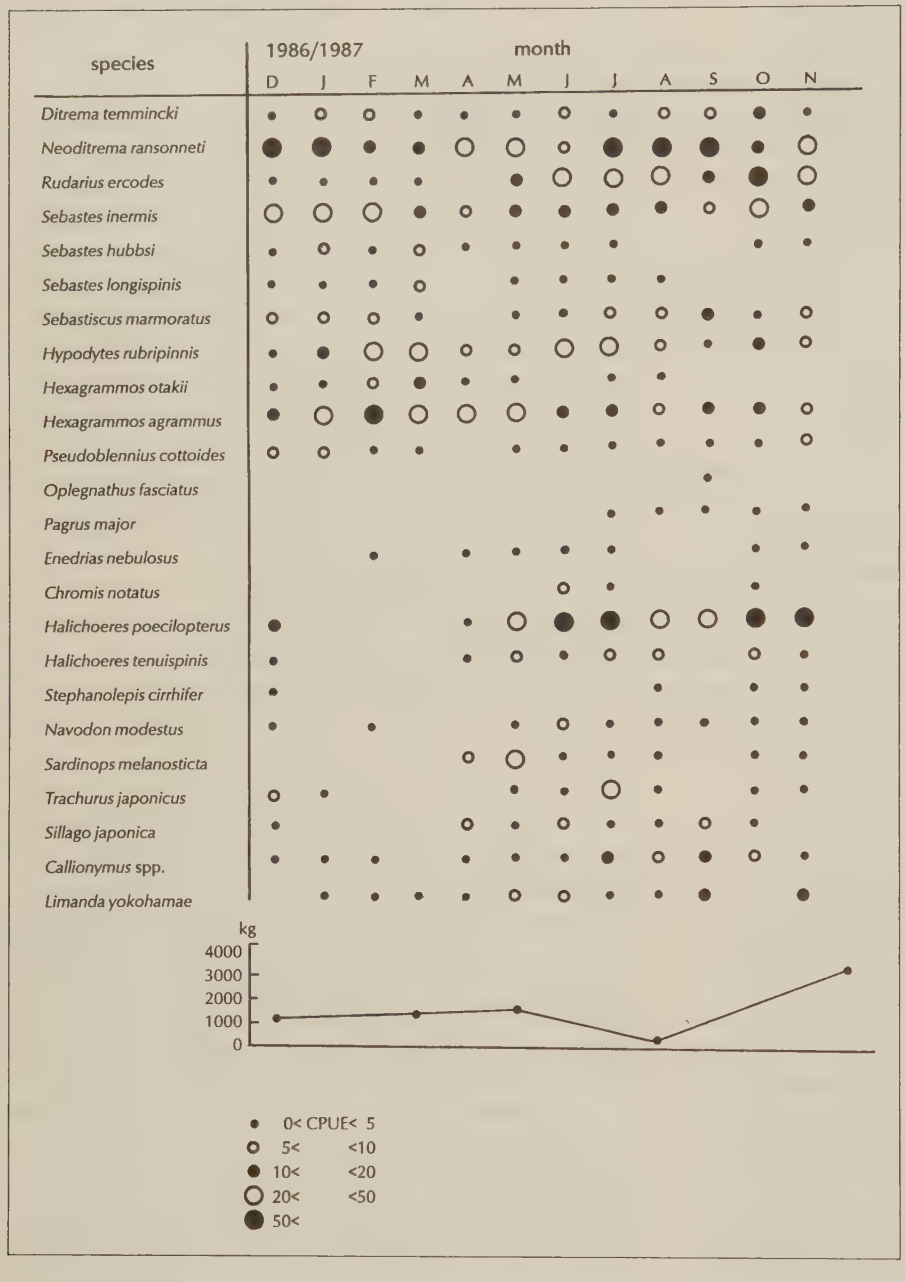

Figure 1
Seasonal appearance of fish species (**upper**) and total amount of seaweed (**lower**) (Yoshikawa and Tsukidate 1987, 1988) in the man-made *Sargassum* forest.

B type, appearing seasonally, including *Halichoeres poecilopterus*, *H. tenuispinis*, *Navodon modestus* (all from spring to autumn) and *Pagrus major* (from summer to autumn); C type, appearing by chance (migratory fish), including *Sardinops melanosticta* and *Trachurus japonicus*; and D type, inhabiting the sandy bottom, including *Limanda yokohamae*. All A and B type species have a close relationship with the seaweed forest. Judging not only from the CPUE but also from the value to commercial fisheries, *S. inermis*, *S. marmoratus*, *H. agrammus*, *D. temmincki*, *H. poecilopterus*, and *N. modestus* are the most important species. They tended to be prevalent throughout the investigations which were conducted from 1983 to 1988 (Okamoto et al. 1987, 1988).

Figure 2 shows the CPUE fluctuations for five of the prominent fish species. Their ecological features are as follows. *S. inermis* juveniles first appear in the spring (Mar.–Apr.); several shoals are observed in some years. Throughout the year, young (0–2 years old) are observed in groups, staying between or above blocks, and beside or among seaweeds. Most of the larger fish (>2 years old) are assumed to migrate to the deeper depths. *H. agrammus* juveniles (type A) settle in the forest during winter (Jan.–Feb.). From the juvenile to the adult stage, they are observed keeping still above the blocks, sometimes picking the seaweeds for cover instead. The CPUE is greatest in winter (Dec.–Mar.) when the quantity of seaweeds in the man-made *Sargassum*

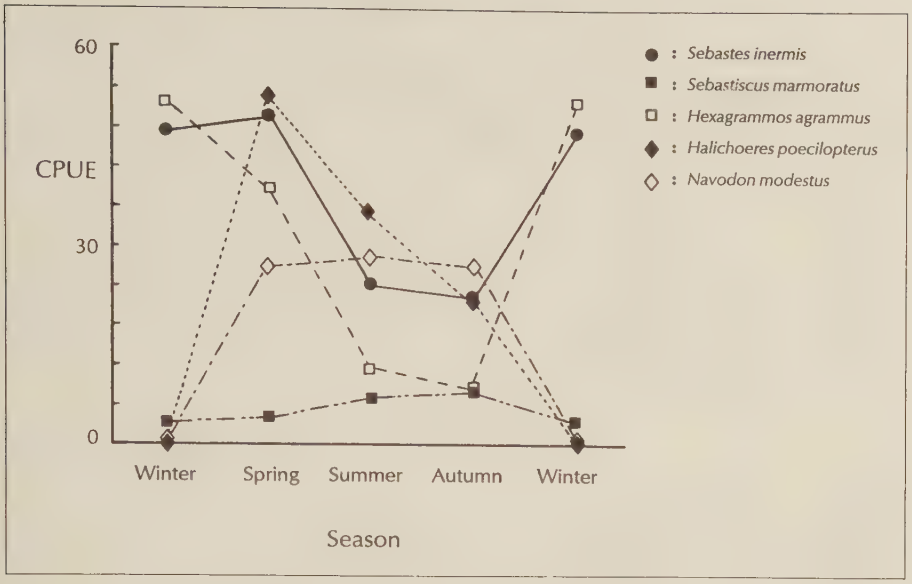

Figure 2
Seasonal fluctuations of CPUE for the main fish species in the man-made *Sargassum* forest (Okamoto et al. 1987).

forest is also at its highest level. Moreover in winter (Dec.–Jan.), many adult females with matured eggs can be captured and clusters of eggs are found attached to seaweeds. *H. otakii* shows similar ecological features, but the CPUE is small. *H. poecilopterus* is a type B species. Increasing spring (Apr.–May) water temperatures awake this species from overwintering to begin activity. Overwintering returns again with the decreased temperatures of winter (Dec.). From the juvenile to the adult stage, it is very active and the CPUE is large. *N. modestus* spawning groups also are B type, coming in spring (Apr.–May), and juveniles appearing in summer (Jul.–Aug.). Both of them stay towards the winter (Nov.–Dec.). In contrast to *H. poecilopterus* overwintering in the sand, they move to the deeper depths to pass the winter. No evidence has been gained to indicate that spawning occurs in the forest, but it seems a good possibility.

Figure 3 shows the presumed amount of available phytal animals in the *Sargassum* forest (per m^2) and the composition of stomach contents in the main fish species during the spring (Mar., Apr., and Jun. 1986) (Matsunaga, unpubl. data). The biomass of the man-made *Sargassum* forest consisted mainly of *S. horneri* which is at its greatest size at that time of year. The amount of phytal animals peaks in March before the forest reaches its maximum biomass level in May. The number and wet weight of phytal animals at this time were 241 (0.48 g) per 1 g wet weight of *S. horneri*, most of which were caprellidian and gammaridian amphipods. One group of the forest (115.2 kg) is estimated to contain 24.8×10^6 (49.1 kg) of phytal animals. In April their levels decrease to 134 (0.20 g) per 1 g of *S. horneri*, and one group of forest (133.2 kg) is estimated to have 17.9×10^6 (27.0 kg) of phytal animals, also dominated by the caprellidian and gammaridian amphipods. In June *S. horneri* begins to wither and cause a sudden decrease in the density of phytal animals to 25 (0.02 g) per 1 g of *S. horneri*, and 3.6×10^6 (2.2 kg) per one group of the forest. Molluscs (mainly bivalves) and polychaetes replaced the amphipods as the predominant animal species.

The stomach contents of *Sebastes inermis* (0–2 years old), *Hexagrammos agrammus*, and *H. otakii* consisted mainly of amphipods in March and April. *Sebastiscus marmoratus* and *Sebastes hubbsi* showed similar trends but the percentages were lower. The reason why *S. inermis* contains both amphipods and copepods is that it takes drifting organisms as food. In June, the ratio and amount of amphipods consumed by the main fish species decreased remarkably, parallel to the level of prey. As mentioned above, main fish species depend on these animals as a source of food, so the man-made *Sargassum* forest should make a large contribution to fish production.

To calculate the extent of which the man-made *Sargassum* forest contributes to fish production, knowledge of species composition, losses due to predation, the reproduction of phytal animals, and other factors are necessary. Presently however, it is impossible to analyze this subject thoroughly. Therefore, only estimations can be carried out. Assuming that the maximum quantity of prey (49.1 kg) undergo complete conversion to fish biomass (conversion ratio of 7.3% from rearing experiments [Matsunaga, unpubl. data], in which fish feed once a day to satisfaction with euphausia), *S. inermis* can increase its own weight by 3.6 kg. If we assume that *S. inermis* gathering in the forest have a daily prey intake rate of 3% of their total body weight, which is the maximum ratio of stomach contents against body weight, then *S. inermis* can increase its own weight by 450 g from March to June. In addition, assuming a food

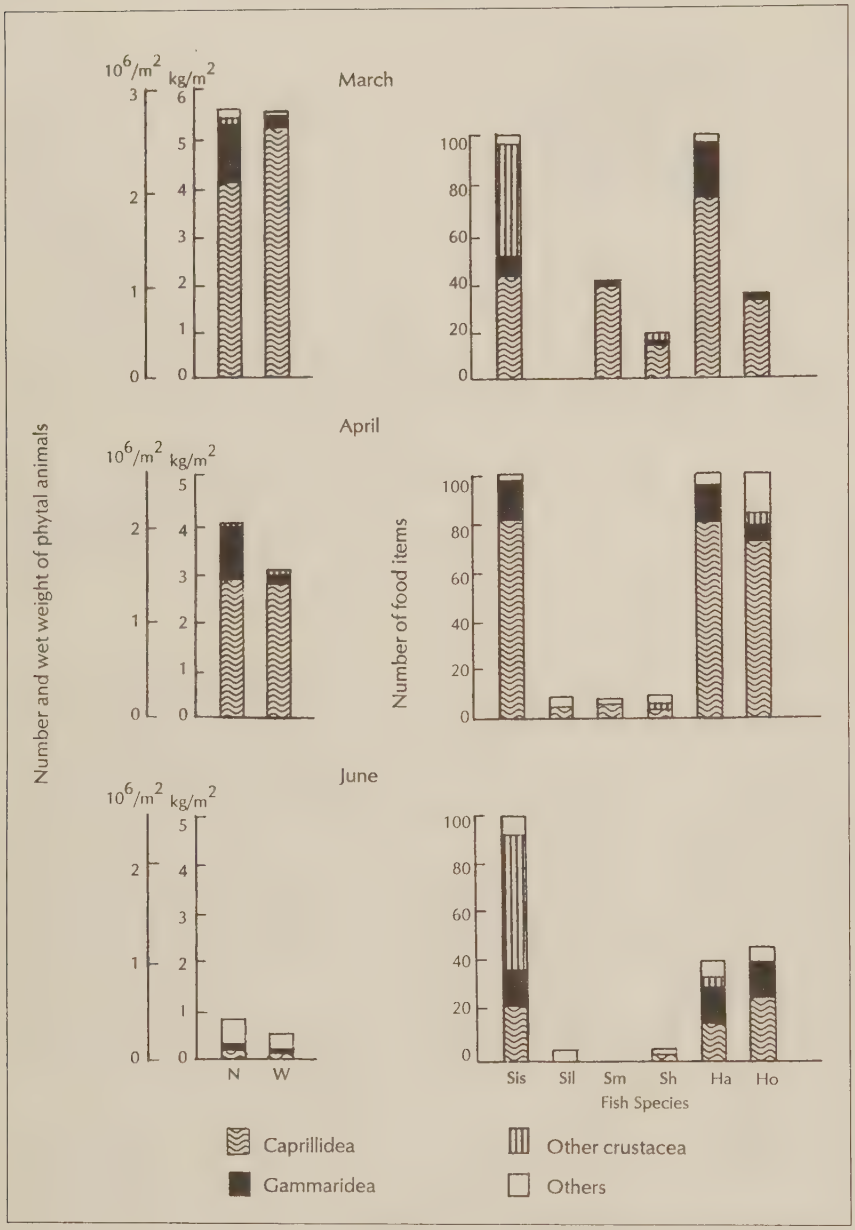

Figure 3

Abundance of phytal animals on one group of the man-made *Sargassum* forest (**left**), and stomach contents of main fish species (**right**) in spring 1986. Where number of food items exceeded 100, the percentage is indicated. N = number; W = wet weight; Sis = *Sebastes inermis* (small); Sil = *S. inermis* (large); Sm = *Sebastiscus marmoratus*; Sh = *Sebastes hubbsi*; Ha = *Hexagrammos agrammus*; Ho = *H. otakii* (Matsunaga, unpubl. data).

intake ratio of 6% which was gained from the rearing experiments, 900 g of fish can be produced.

These evaluations, which were carried out to study the effect of man-made *Sargassum* forests on fish production, are necessary to accumulate detailed knowledge to support the planned enlargement of the coastal fisheries resource.

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Masu Salmon Production Studies of the Marine Ranching Program

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ABSTRACT

Results from the masu salmon (*Oncorhynchus masou* Brevoort) production studies of the Marine Ranching Program from 1980 to 1988 are reviewed in the present paper. Seeking to ensure adequate production levels of this valuable fishery resource under ever increasing amounts of man-made stress, Japanese scientists have examined important aspects of masu salmon biology and utilized artificial means to supplement the natural stock levels. Using techniques successfully developed for the chum salmon (*O. keta*), naturally ascending masu salmon were similarly captured and reared under freshwater conditions without food throughout their extended freshwater migration period of four months, until reaching full maturation. Over 80% of the pond-reared fish reached full maturity, providing an excellent source of eggs and sperm for enhancement (seedling production) programs and other studies. Serum concentrations of steroid hormones were measured in addition to their ability to artificially induce germinal vesicle breakdown *in vitro*. Other research discussed includes all-female seedling production, migration patterns, smolt production and liberation studies, net-pen culturing, resources analysis, and important genetic features of the masu salmon population. These developments have improved artificial propagation methods and thus resulted in the large scale acceleration of masu salmon resources.

Introduction

Almost all the salmon resources in Japan have been supported by artificial propagation. Four species constitute the bulk of these programs in Japan, namely, chum salmon (*Oncorhynchus keta*), pink salmon (*O. gorbuscha*), masu salmon (*O. masou*), and Kokanee (*O. nerka*). In recent years, chum salmon resources have risen steadily, to about 160 thousand tons. This noticeable increase is due to the application of studies conducted on the mass production of chum salmon from 1977 to 1981 by the Agriculture, Forestry, and Fisheries Research Council Secretariat.

Like chum salmon, masu salmon are one of the important coastal fishery resources in Japan. In recent years, however, the level of masu salmon resources has decreased markedly, to about two thousand metric tons. Masu salmon have long freshwater residency periods during both the fry to fingerling stage and the anadromous migration. A large number of male fish remain in the rivers and accomplish their life cycle without travelling to sea. To increase this resource, it is important to model the natural behaviors of these fish, especially those concerned with growth, smoltification, downstream migration, feeding migration, sexual maturation, and anadromous migration.

Masu salmon production studies under the Marine Ranching Program have been conducted from 1980 to 1988 by the Agriculture, Forestry, and Fisheries Research Council Secretariat, focusing on the subjects of sexual maturation, seedling production, smoltification, nutrition, stream production capacity, smolt-liberation, all female production, seaward-migration, fish disease, pen-culture, resources analysis, genetic features, and imprinting of home stream water. This paper reviews some of the accomplishments of Japanese scientist seeking to improve artificial propagation methods to increase this valuable resource.

Sexual Maturation

Maturation in masu salmon progresses synchronously in the testicular lobules of males (Hiroi and Yamamoto 1970) and in the ovarian oocytes of females (Hiroi 1984). As with the other species belonging to the genus *Oncorhynchus*, male and female masu salmon are destined to die after spawn-

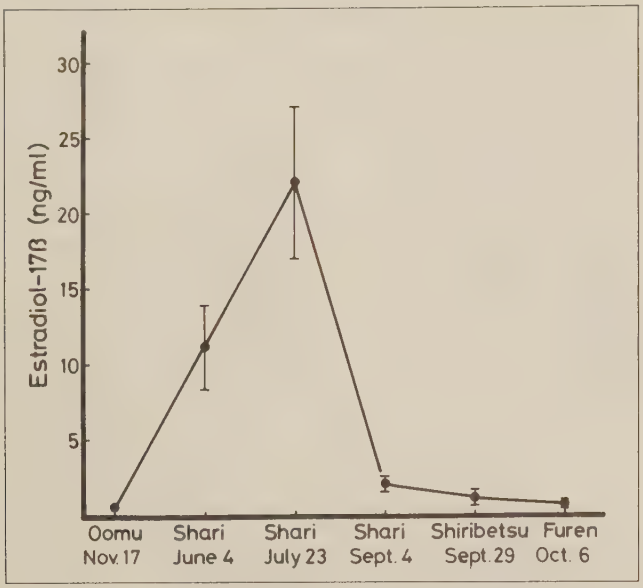


Figure 1

Changes in serum estradiol-17β levels during sexual maturation in female masu salmon. **Oomu** coast (17 Nov.), immature fish (just over 2 years old) caught in the coastal sea; **Shari** River (4 June and 23 July), maturing fish (3 years old) reared in a holding pond after being caught near the river-mouth; **Shari** (4 Sept.), **Shiribetsu** (29 Sept.), and **Furen** (6 Oct.) Rivers, mature fish (3 years old) from a holding pond (Hiroi et al. 1983).

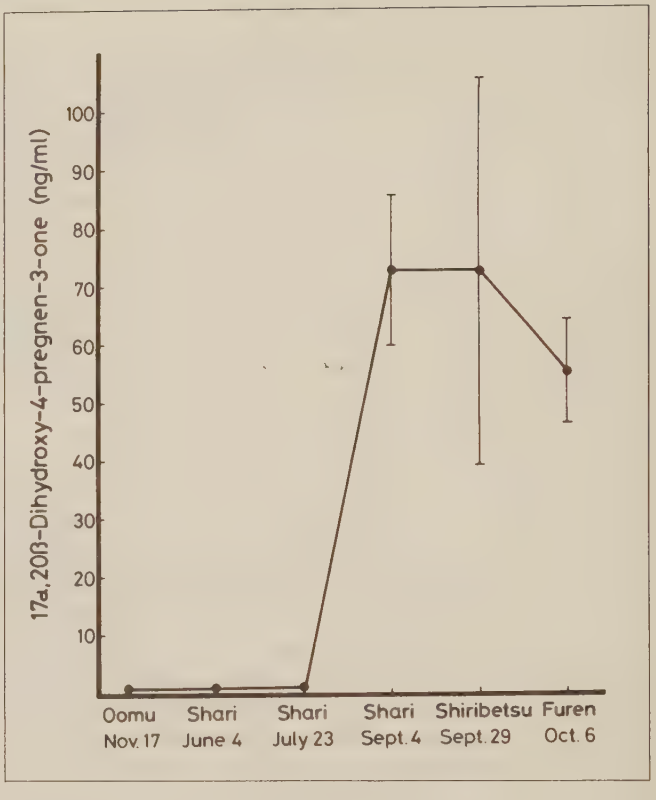


Figure 2

Changes in serum 17α,20β-dihydroxy-4-pregnen-3-one levels during sexual maturation in female masu salmon. **Oomu** coast (17 Nov.), immature fish (just over 2 years old) caught in the coastal sea; **Shari** River (4 June and 23 July), maturing fish (3 years old) reared in a holding pond after being caught near the river-mouth; **Shari** (4 Sept.), **Shiribetsu** (29 Sept.), and **Furen** (6 Oct.) Rivers, mature fish (3 years old) from a holding pond (Hiroi et al. 1983).

ing, the final step of their anadromous migration to the home river from the far seas.

As they return to their natal river from the sea in late April, masu salmon are extremely immature sexually (Hiroi 1984; Kiso and Kosada 1988). They ripen from late August to September, about four months after entering the rivers. Thus, their sexual maturation proceeds rapidly during the final period of their anadromous migration (Hiroi 1984).

In order to clarify the endocrine factors related to sexual maturation, serum concentrations of various steroid hormones were measured with radioimmunoassay techniques during the anadromous migration of male and female masu salmon (Hiroi et al. 1983).

A total of 78 fish (26 males, 52 females) were captured at sea or in freshwater in 1982 and used in the present study. Twenty-five of these fish were just over 2-years-old when caught in the coastal sea off Oomu in November. At this stage, both sexes were extremely immature sexually. The 12 males were in the late multiplication stage of testicular maturity (by histological observation, Hiroi and Yamamoto 1970) and averaged 0.07% on the gonadosomatic index (GSI, gonad wt./body wt. × 100). The remaining 13 females were in the oil-drop stage of ovarian maturity (by histological observation, Hiroi 1984, 1985; Yamamoto 1970) and averaged 0.5% on the GSI. Twenty-seven sexually maturing adults were sampled in June and July from fish being reared in holding ponds after their capture near the mouth of the Shari River at age-3. Thirteen females sampled in June were in the primary yolk stage (2.9%, mean GSI) and four fish taken in July were in the post-migratory nucleus stage (18.3%, mean GSI). Male fish sampled in June and July (five each month) showed the early (1.0%, mean GSI) and late (8.4%, mean GSI) phases of the sperm-formation stage, respectively. Twenty-six fish sampled from the holding ponds at the Shari River (early September), the Shiribetsu River (late September), and the Furen River (early October) were all fully mature. The mean GSI values of mature females in the Shari (9 fish), Shiribetsu (6 fish), and Furen River (7 fish) were 27.3%, 21.8%, and 24.2%, respectively. The mean GSI value of mature males in the Shiribetsu River (4 fish) was 3.7%.

In female fish, serum estradiol-17β levels were high midway through vitellogenesis (June and July in the Shari

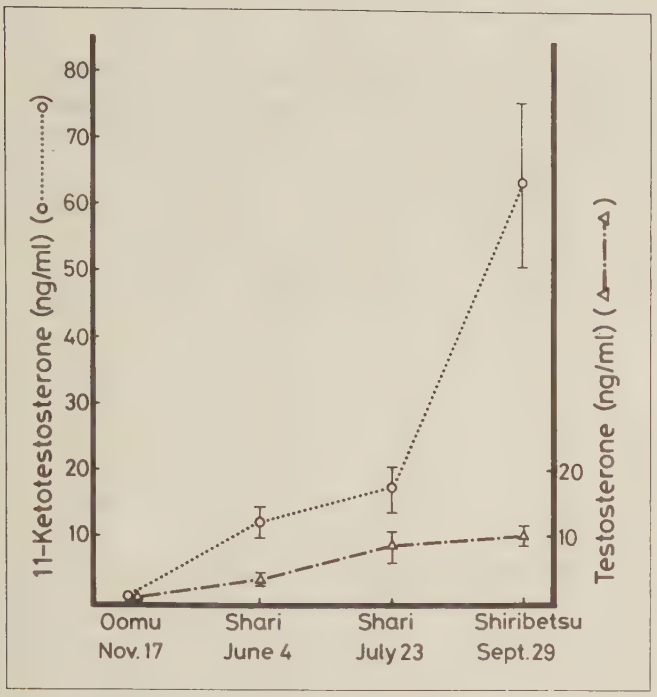


Figure 3

Changes in serum 11-ketotestosterone and testosterone levels during sexual maturation in male masu salmon. **Oomu** coast (17 Nov.), immature fish (just over 2 years old) caught in the coastal sea; **Shari** River (4 June and 23 July), maturing fish (3 years old) reared in a holding pond after being caught near the river-mouth; **Shiribetsu** River (29 Sept.), mature fish (3 years old) from a holding pond (Hiroi et al. 1983).

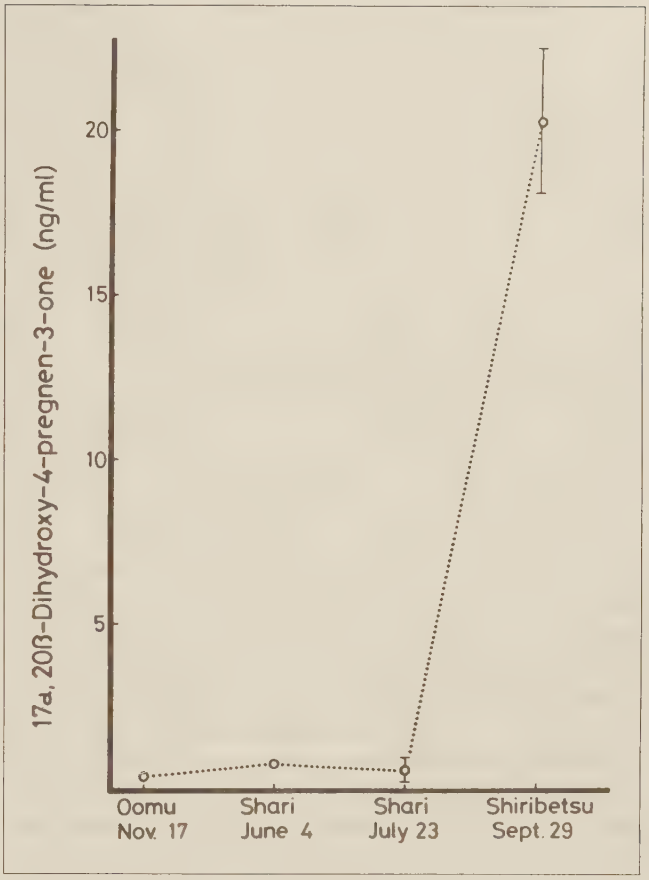


Figure 4

Changes in serum 17α,20β-dihydroxy-4-pregnen-3-one levels during sexual maturation in male masu salmon. **Oomu** coast (17 Nov.), immature fish (just over 2 years old) caught in the coastal sea; **Shari** River (4 June and 23 July), maturing fish (3 years old) reared in a holding pond after being caught near the river-mouth; **Shiribetsu** River (29 Sept.), mature fish (3 years old) from a holding pond (Hiroi et al. 1983).

River) but significantly lower in fish at the time of final maturation (September: 2 samplings, one in each of the Shari and Shiribetsu Rivers; and October: 1 sampling, in the Furen River) (Fig. 1). These results provide strong evidence that estradiol-17β plays a major role in the synthesis of vitellogenin in female masu salmon as seen in chum salmon (Hiroi 1982, 1985; Ueda et al. 1984).

Serum 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-diOHprog) levels were extremely low in female masu salmon prior to migration from the sea (in November off the Oomu coast) and halfway through vitellogenesis (June and July, Shari River), but elevated dramatically with the onset of ovulation (September: 2 samplings, one in each of the Shari and Shiribetsu Rivers; and October: 1 sampling, in the Furen River) (Fig. 2). This provides strong evidence that 17α,20β-diOHprog is the natural maturation-inducing steroid involved in final oocyte maturation as seen in chum salmon (Hiroi 1982, 1985; Ueda et al. 1984).

In males, serum testosterone and 11-ketotestosterone levels were extremely low in sexually immature fish at sea (November in the Oomu coast) but elevated gradually in the maturing fish in freshwater (June and July, both samples from the Shari River; through September in the Shiribetsu River) as spermatogenesis progressed (Fig. 3). This observation suggests that testosterone and 11-ketotestosterone are involved in the process of spermatogenesis in males as seen in chum salmon (Hiroi 1982, 1985; Ueda et al. 1984).

Serum 17α,20β-diOHprog levels, although lower in males, increased rapidly in mature males (Fig. 4) as previously seen in females. This observation provides strong evidence that 17α,20β-diOHprog is involved in the process of spermiation as seen in chum salmon (Hiroi 1982, 1985; Ueda et al. 1984).

The possibility of artificially inducing maturation *in vitro* using chum salmon gonadotropin (SGA) and various steroid hormones to induce germinal vesicle breakdown (GVBD) of oocytes was investigated. Oocytes (444 g in the total weight) were removed from maturing female (2,080 g

in body weight) captured in the middle reaches of the Shiribetsu River while in the late vitellogenesis phase, about 15 days before ovulation (Hiroi, Ueda, Yamauchi and Nagahama, unpubl. data).

Twenty oocytes were incubated *in vitro* for 72 hours at 15°C in various doses of hormone supplemented ringer solutions. Although the oocytes were not changed at all by the incubations of ringer control and testosterone (Table 1), oocyte maturations (GVBD) were induced by the incubations of over 0.1 μg/mL doses of SGA, 1 μg/mL dose of 17α-hydroxy-4-pregnen-3-one (17α-OHprog; a substrate for 17α,20β-diOHprog biosynthesis) and over 0.01 μg/mL doses of 17α,20β-diOHprog. These results show that it is possible to induce GVBD artifically (but not ovulation) by *in vitro* incubations of 17α,20β-diOHprog or using SGA in maturing oocytes of the late vitellogenesis phase in masu salmon as previously seen in chum salmon (Hiroi 1982, 1985). In testosterone treatments, a change to a migrated nucleus in the oocytes was observed only in the 1.0 μg/mL incubation. Serum testosterone levels in female masu salmon were high throughout the course of anadromous migration, showing the highest value in mature fish (Hiroi et al. 1983) as seen in chum salmon (Hiroi 1982, 1985; Ueda et al. 1984). The close relationship observed between estradiol-17β and testosterone levels during late vitellogenesis in female masu salmon supports the suggestion that testosterone may act as a substrate for estradiol-17β biosynthesis, but the precise roles of testosterone in oocyte maturation are presently unknown.

Table 1

Effects of chum salmon gonadotropin (SGA), 17α-OHprog, 17α,20β-diOHprog, and testosterone on the induction of GVBD of masu salmon oocytes *in vitro* (Hiroi, Ueda, Yamauchi, and Nagahama, unpubl. data).

Treatment		Oocyte condition (% ± SEM)		
Hormone	Dose (μg/mL)	GVBD	Migrated nucleus	Immature
SGA	1.0	78 ± 2	22 ± 2	0
	0.1	25 ± 5	75 ± 5	0
	0.01	0	48 ± 2	52 ± 2
17α-OHprog	1.0	100	0	0
	0.1	0	100	0
	0.01	0	72 ± 8	28 ± 8
	0.001	0	35 ± 20	65 ± 20
17α,20β-diOHprog	1.0	80 ± 5	20 ± 5	0
	0.1	80 ± 5	20 ± 5	0
	0.01	58 ± 2	42 ± 2	0
	0.001	0	50 ± 5	50 ± 5
Testosterone	1.0	0	100	0
	0.1	0	0	100
	0.01	0	0	100
	0.001	0	0	100
Ringer control		0	0	100

Seedling Production

Seedling production was investigated using artificially collected eggs taken from the naturally migrating, pond-cultured, and pen-cultured adults.

Use of the naturally migrating adults required holding the sexually maturing fish in freshwater ponds for a long period of about four months. It is known that the source of energy for ripening of masu salmon during the long period of abstinence from food is the neutral fat which the fish have stored in great quantity during their oceanic life (Nomura 1984; Nomura et al. 1985).

Five factors describe the basic conditions considered necessary for the long-term holding of sexually maturing masu salmon taken from rivers. First, the captured fish must be held in their specific home stream waters because of their instinctive homing nature. Second, we have to understand the seasonal changes in the degree of sexual maturation of adults soon after entering each river or catching station. Knowledge of the degree of maturation is necessary to determine the optimum time for mature fish selection for the collection of eggs. Because of their extremely immature condition as they enter the rivers, it is important for us to improve fish handling methods in order to prevent stripping of scales at the time of capture and transport. Third, the holding waters for the maturing adults must be kept within an optimum temperature range of 8 to 12°C. This can be achieved by mixing spring water with the river water. Fourth, water currents in the holding ponds must be kept slow (under 10 cm/sec) to mitigate exhaustion of fish from over-swimming. For masu salmon operations, holding or rearing ponds with the water supply welled up from the bottom and with periodic exchanges of new water are most suitable. Fifth, covering the pond surface from light is essential for resting the fish.

Using a method meeting those basic conditions, long-term holding experiments with spring water were carried out on a large-scale with migrating masu salmon from 1983 to 1988 (Table 2). The sexually immature adults (about 1,100 g in weight ranging from 230 to 2,0000 g) were collected every year from late April to late May at the catching station near the mouth of the Shari River. The fish were transferred from a catching pool to a transport car, together with water, using a "fish ladle." The ladle's net was made of duck-cloth and had several small holes; this special net prevented the stripping of scales. Every morning and evening fish travelled in the car for about one hour from the catching station to the holding pond site where an immediate transfer occurred. The eight holding ponds reformed for these experiments were made of concrete (5 m wide, 25 m long, and 0.9 m deep), and had an up-welled current of spring water (1 m^3/min) created by

Table 2
Long-term holding experiments with spring water on naturally ascending masu salmon, 1983–1988. Fish were held without food for duration of experiment.

Year	Number of females	Duration of holding (days)	Rate of maturation (females) (%)	Number of eggs collected (thousands)	Average number of eggs collected per fish
1983	517	125–133	43	238	1,301
1984	1,169	110–135	77	1,082	1,213
1985	767	103–136	84	744	1,380
1986	859	105–135	82	858	1,222
1987	343	100–126	88	354	1,172
1988	671	102–139	86	745	1,321

flowing water one way from the upper third portion of the pond bottom. Ponds were always covered by cheesecloth-like black sheets to exclude light. Immature fish were held at a density of 600 fish per pond (75 m^3 of water volume) without food for four months at a water temperature of 9 to 10° C. Recently, over 80% of the naturally ascending masu salmon reached full maturity in the holding ponds under these basic conditions (Table 2).

Egg and sperm collected in early autumn from mature fish were stirred gently and inseminated using a dry method. Fertilization, namely the activation of eggs, was completed by contact of inseminated eggs with water. Fertilized eggs were then washed with up-welled currents and put in a hatchery trough.

The artificial production of seedlings from the collected eggs of both pond-cultured and pen-cultured adults has been carried out easily for several generations (Hiroi 1984; Yoshida et al. 1987).

Smoltification

Although the mechanism of smoltification has not yet been fully understood, clearly there is a close correlation between smoltification and the fish's adaptability to sea water and between the functions of the thyroid gland and interrenal gland hormone (Yamazaki and Ma 1985; Yamazaki 1986). The process of the silvering of body coloration during smoltification was induced by treatments of mammalian thyroid hormone powder; however, seawater adaptability was not accelerated by cortisol treatments (Yamazaki 1983).

Nutrition

Nutrition of the masu (cherry) salmon was studied by Ogata and Konno (1986). The authors determined that nonpolar lipid contents in the whole body, dorsal muscle, and liver of freshwater smolt (approx. 2 years old) are lower than those of the parr (1 year old). During March through May, nonesterified fatty acid levels in the plasma of the smolt were higher than those of the parr, but the neutral fat levels in the smolt were lower.

The growth response and smolt production of 1-year-old masu salmon were studied by feeding the fish four diets with varying lipid and protein levels for 120 days. Each of the following dietary treatments were administered to 55 fish averaging 16 g: HPHL, high protein (41%)-high lipid (16%); HPLL, high protein-low lipid (4%); LPHL, low protein (24%)-high lipid (14%); LPLL, low protein-low lipid (2%). The growth response to these dietary treatments was as follows: HPHL (final mean body weight, 44.4 g)>HPLL (36.5 g)>LPHL (29.0 g)>LPLL (28.0 g). Only in the high protein diets did both growth rate and feed efficiency clearly improve by the addition of lipid (Table 3). In all treatment groups, the growth, feed efficiency, and feed consumption rate accelerated with the onset of smoltification. Percentage of smolt produced were 74.5% in HPHL, 47.3% in HPLL, 29.1% in LPHL, and 16.4% in LPLL, respectively. Thus, supplementation of lipid to diet seems to be a valuable method to promote smolt production.

Stream Production Capacity

Environmental devastation in rivers caused by land and industrial developments in recent years have resulted in serious decreases in stream production capacity which is affecting the survival of masu salmon from the fry to the juvenile stage. Marked masu salmon fry (167 thousand fish) released experimentally in the upstream areas of the Mena River (a tributary of the Shiribetsu River) during the spring, have been collected only at the lower stream areas, not at the upper areas a month after the release

Table 3

Response of 1-year-old masu salmon to various dietary lipid and protein levels in a 120-day feeding experiment (Ogata and Konno 1986). HPHL = high protein, high lipid; HPLL = high protein, low lipid; LPHL = low protein, high lipid; LPLL = low protein, low lipid. Each tank contained 55 fish.

	Diet							
	HPHL		HPLL		LPHL		LPLL	
	0–89	90–120	0–89	90–120	0–89	90–120	0–89	90–120
Average body weight (g)								
Initial	15.49		15.83		16.69		16.06	
Final	29.97	44.41	26.83	36.47	23.41	29.04	22.22	28.02
Daily growth rate (%)	0.74	1.27	0.59	0.99	0.38	0.69	0.36	0.75
Feed efficiency (%)	55.50	65.10	41.50	56.70	24.80	40.40	23.60	32.90
Protein efficiency ratio (%)	1.23	1.45	0.92	1.26	0.99	1.61	0.94	1.32
Daily feed consumption rate (%)	1.29	1.92	1.25	1.73	1.19	1.71	1.60	2.26
Protein intake[3] (g/kg fish each day)	5.81	8.64	5.63	7.79	2.98	4.28	4.00	5.65
Number of smolt obtained*	42		26		16		9	
Average body weight of smolt (g)	43.57		39.59		30.39		34.73	

*Difference ($P<0.01$) can be found among the dietary treatments by χ^2 test on the number of smolt obtained.

(Ohkuma and Nomura 1991). Consequently, a great number of masu salmon fry, as well as chum salmon fry, need to be scattered at the uppermost part of the streams for the industry to effectively utilize the potential productivity of a river.

The successful release of fingerlings (less than 1 year old) in the autumn has been confirmed from the return of marked masu salmon adults by Mayama et al. (1988).

Smolt-Liberation

Concerning production of greater than 1-year-old (1^+) smolt, it is known that the external control of young masu salmon growth during each development stage is practical (Mayama et al. 1986). This includes first, initial control to curb sexual maturation of fingerling males (precocious males); second, the acceleration of growth during the parr stage; third, inhibition of growth through the winter season; and last, the acceleration of growth prior to smoltification. A diagrammatic illustration of growth control in the smolt-liberations of masu salmon fingerlings is shown in Figure 5. Control of smoltification was achieved by appropriately combining and adjusting water temperature, light cycle periods, and the quantity of feed. These artificial controls result in over 90% conversion using growth controls during the fry to fingerling period. Recently, the return of adult masu salmon cultured by the methods of smolt-liberation described above has been ascertained. A high return rate of over 7% was estimated by the recapture of marked fish (Mayama et al. 1985).

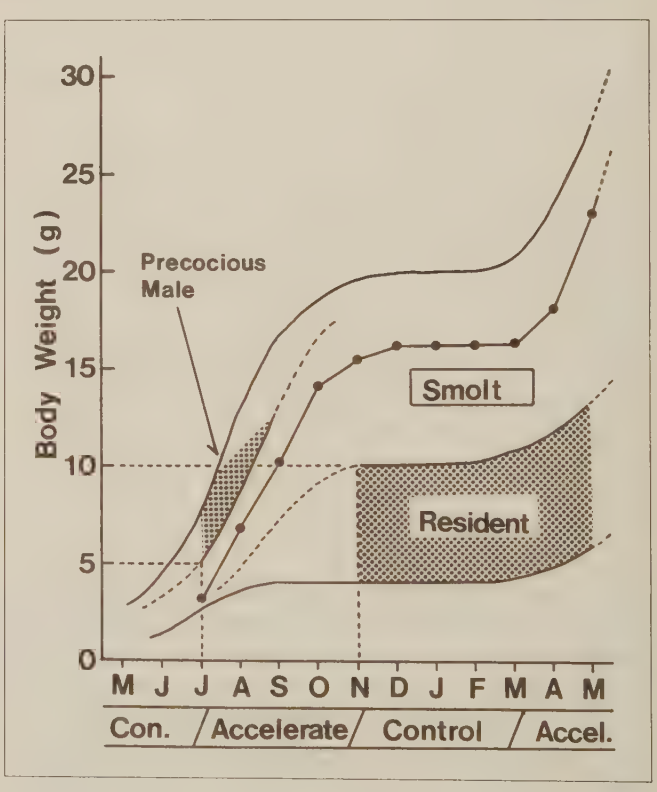


Figure 5

Diagram showing growth control in the enhancement of smolt masu salmon (age 1^+). Growth curve with solid dots indicates the average body weight standard for rearing (Mayama et al. 1986).

The duration of the period that masu salmon spend in freshwater prior to smoltification can be reduced to half

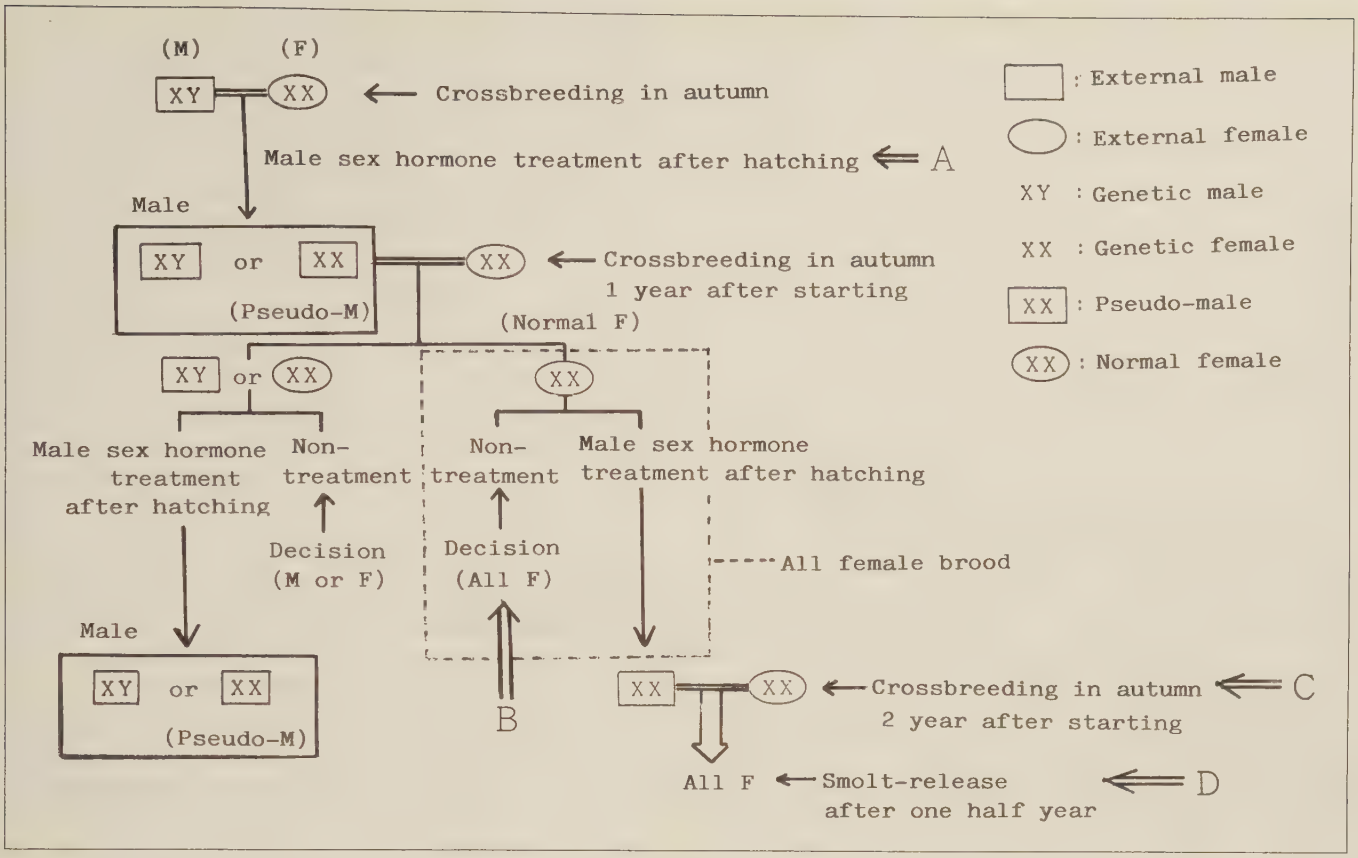


Figure 6
Schematic illustration of the production technology of producing all-female brood in masu salmon (Kanazawa and Harako 1985).

that of the normal life cycle (0^+ smolt). It is necessary to obtain eggs earlier by artificial means (thereby hastening production of the young) and then to rear the fish in higher-than-ambient water temperatures (13–15°C) during the winter season. Earlier egg-taking in the summer has been accomplished by methods that control light exposure to the parent held in a pond strain (Konno et al. 1983). Returns of age 0^+ smolt raised from the parent pond strain to home waters have been confirmed in the Nezugaseki River in Yamagata Prefecture (Konno and Abe 1987). Although only three returning fish were confirmed, it is thought that releasing age 0^+ smolt in the spring season is as effective as that of age 1^+ smolt.

All-Female Production

All-female fish production, as a means of making smolt production more efficient, can be carried out by cross-breeding between normal returning females and "psudo-males" (the latter are genetic females), as shown in Figure 6 (Kanazawa and Harako 1985). Pseudo-males are produced by immersion treatments of normal female fry of masu salmon in solutions of the male sex hormone, 17α-methyl-testosterone. Pseudo-males tend to form improperly developed vas deferens, and produce sperm with female genetic characteristics. Adult returns of the feminized, marked fish have been ascertained in home rivers (Kanazawa and Harako 1987). Though this technology has not yet reached a mass production stage, it can be said that this type of smolt production technology has almost been established.

Seaward-Migration

Seaward-migrations of smolt occur actively from early April to early May in the second spring of development, with the fish moving to the coastal sea until late May. The smolts entering the sea grow rapidly, feeding upon the abundant food available in coastal waters. Their main diet at that stage is small fish (Harako 1983; Kato 1983; Kemuyama et al. 1987; Kiso 1986, 1987; Miyazawa et al. 1986).

The overwhelming percentage of seaward migrating fish are female (60 to 96%), indicating that the majority of male masu salmon juveniles will remain in rivers (Kato 1983;

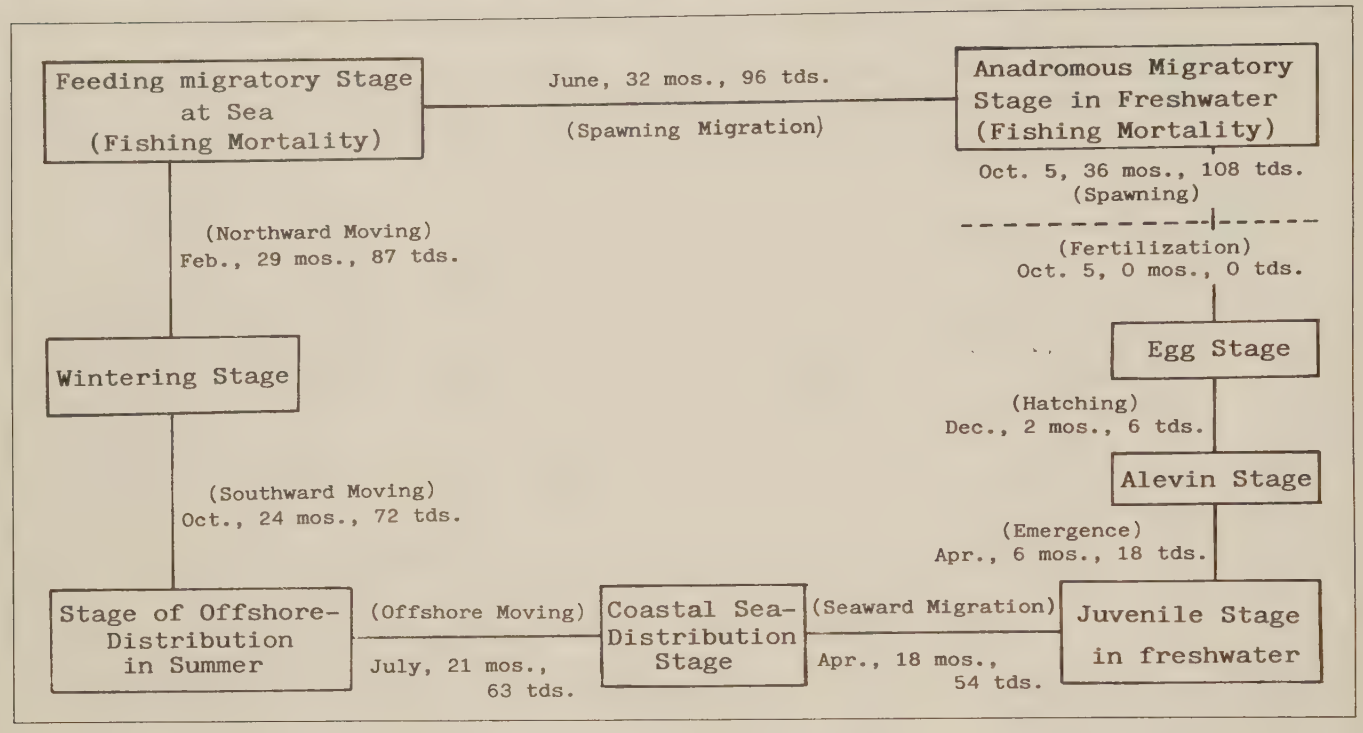


Figure 7

A typical model of the life cycle in masu salmon with calendar month, age in months (mos.) and age in ten-days (tds.) after fertilization (Kato and Hasegawa 1986).

Kemuyama et al. 1987; Kiso 1986; Miyazawa et al. 1986; Ohkuma 1988; Sasaki et al. 1987). Remaining males in rivers are explained by the observations that the maturation of precocious male parr depends greatly upon the growth of fish during the fry to fingerling stage (Utoh 1976) and that the body lipid content of precocious male parr is higher than that of smolt soon after the seaward-migration, showing declines of body lipid content with the smoltification (Nomura 1984; Nomura et al. 1985).

Pen-Culture

Growth size of the masu salmon juveniles at the beginning of pen-culture requires an initial body weight of over 130 g by mid-November to ensure proper seawater tolerance (Yoshida et al. 1987). Pen-culture adults grow to about 1,000 g in weight, showing a noticeable delay of growth in the summer after late June.

Resource Analysis

Stock assessment of the Japan Sea masu salmon population was calculated statistically from the relationship between the total catch and total spawns (Kato and Hasegawa 1986). A typical masu salmon life cycle segmented into ten-day intervals was chosen for simplifying the statistical analyses of the resources (Fig. 7). The growth (average fork lengths) of adult masu salmon captured by the drift nets of administrative ships from 1973 to 1980 was expressed using von Bertalanffy's growth curve:

$$L_t = 54.3758\ (1 - e^{-0.1192(t - 77.1523)}), \qquad (1)$$

where t was age (in ten-day units) after fertilization and L_t was the fork length at t ten-day units after fertilization (Fig. 8). A relationship between the fork length (L, cm) and the body weight (W, g) of adults, using the findings from fish captured in the coastal sea off Ishikawa Prefecture in March of 1979, was shown by the following equation:

$$W = 0.014108L^3. \qquad (2)$$

The masu salmon life span is 3 years (108 ten-day units) with the exception of only a few fish (Ohkuma 1988). It was assumed that the sex ratio of the fish was 1:1, the average spawn was 1,945 eggs per female, and the average weight of the total annual catch in the Japan Sea was about 3,000 metric tons. The fishing mortality numbers (which corresponded with age composition) calculated from the number of fish caught per roll of drift net in each of the ten-day periods were obtained from the findings of the drift net fishery from 1968 to 1980 in the Japan Sea. From this

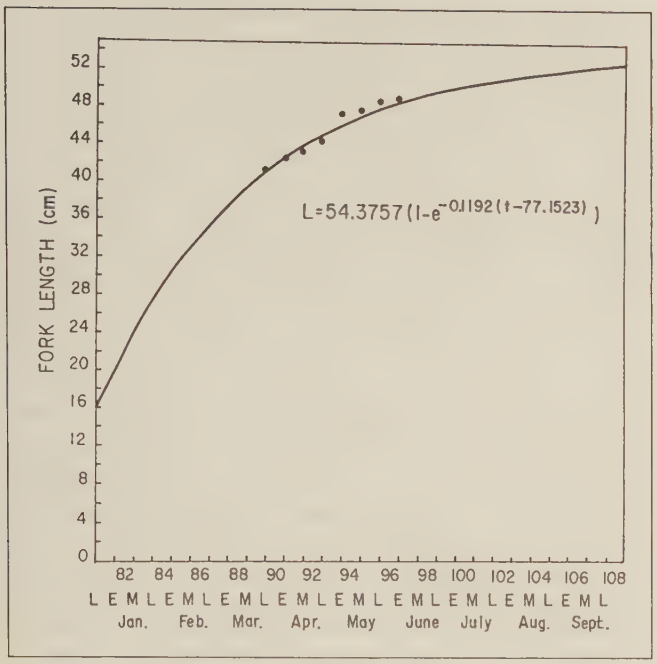

FORK LENGTH (cm)
L=54.3757(1-e^-0.0192(t-77.1523))
82 84 86 88 90 92 94 96 98 100 102 104 106 108
L E M L E M L E M L E M L E M L E M L E M L E M L E M L
Jan. Feb. Mar. Apr. May June July Aug. Sept.

Figure 8

Growth curve of masu salmon adults with both calendar month and age (in ten-day units) after fertilization, using von Bertalanffy's equation (Kato and Hasegawa 1986).

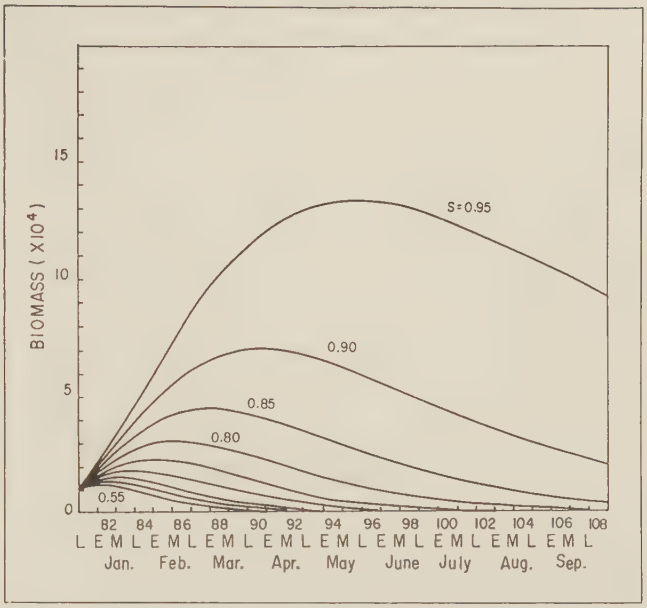

BIOMASS (X10⁴)
S=0.95
0.90
0.85
0.80
0.55
82 84 86 88 90 92 94 96 98 100 102 104 106 108
L E M L E M L E M L E M L E M L E M L E M L E M L E M L
Jan. Feb. Mar. Apr. May June July Aug. Sep.

Figure 9

Curves of relative weight of the virgin (absence of fishing mortality) masu salmon resource (biomass; g), as the survival rate of fish (S) varies from 0.55 to 0.95, by calendar month (E = early, M = middle, L = late part of month) and age (in ten-day units) after fertilization. The weight at 80 ten-day units (late December) is 10,000 g (Kato and Hasegawa 1986).

it was shown that the fish became available to the masu salmon fishery starting in early March (87 ten-day units).

The masu salmon adults, as shown in Figure 7, seem to begin the wintering stage in December of their third year (approximately 25 months) to decrease their natural mortality and stabilize their survival rates. The number of virgin (or number in the absence of fishing mortality) masu salmon, N_x, at X ten-day units are estimated by the equation

$$N_x = N_0 S_0^{(X-80)},$$

where N_0 is numbers of fish at 80 ten-day units (late December) and S_0 is the survival rate per ten-day unit. Accordingly, the weight of a virgin resource fish, P_x, in X ten-day units is calculated by the equation

$$P_x = N_x W_x = W_x N_0 S_0^{(X-80)},$$

where W_x is the weight of a fish at X ten-day units obtained from Equations 1 and 2. The approximate weight of a virgin resource fish at 80 ten-day units, P_0, is 10,000 g. Weight after 80 ten-day units can then be plotted against the survival rate of the fish from 0.55 to 0.95 (per ten-day unit) resulting in the curves of relative resources weight shown in Figure 9. Because masu salmon have fewer eggs per spawn than other fish, the survival rate (S_0) of the virgin resources seems to be near 0.95 (Fig. 9). Accordingly, the total mortality coefficient, Z, of the virgin resources calculated by the equation

$$Z = \ln(S_0) = -\ln(0.95) = 0.051 = M \qquad (3)$$

can be regarded as the natural mortality coefficient, M.

The fishing resources to decrease from early March (87 ten-day units) to middle June (97 ten-day units). The survival rate of the resources experiencing fishing mortality seems to be 0.85, which results in a total mortality coefficient (Z) of 0.162 per ten-day unit from Equation 3. Accordingly, fishing mortality coefficient, F, can be presumed to be 0.111 (total mortality–natural mortality). Then, the fishing rate (in fish per ten-day units), E, is calculated as 0.103 from the equation

$$E = F\,[1 - e^{-(F+M)}/(F+M)].$$

From the coefficients and rates mentioned above, numbers of fish and numbers of eggs (spawns) in both the virgin resources and the resource under fishing mortality pressure can be calculated for each of the ten-day periods.

To determine the optimum starting time for the fishery in relation to fishing intensity, isopleth curves of both the weights of the total annual catch and the decreased percentage of total spawns (number of eggs) are plotted against

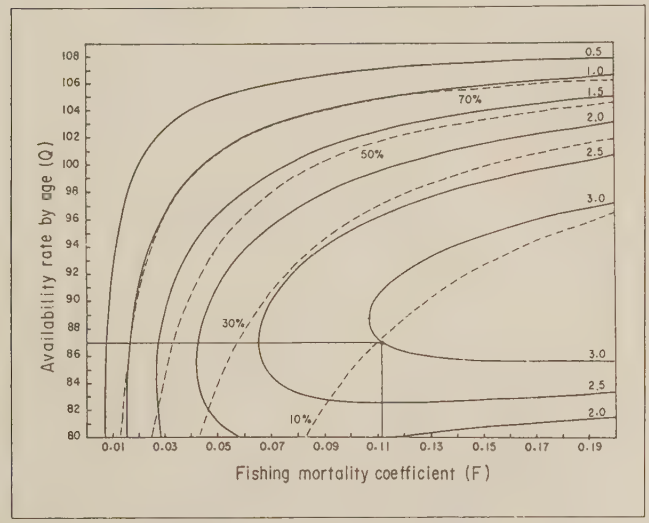


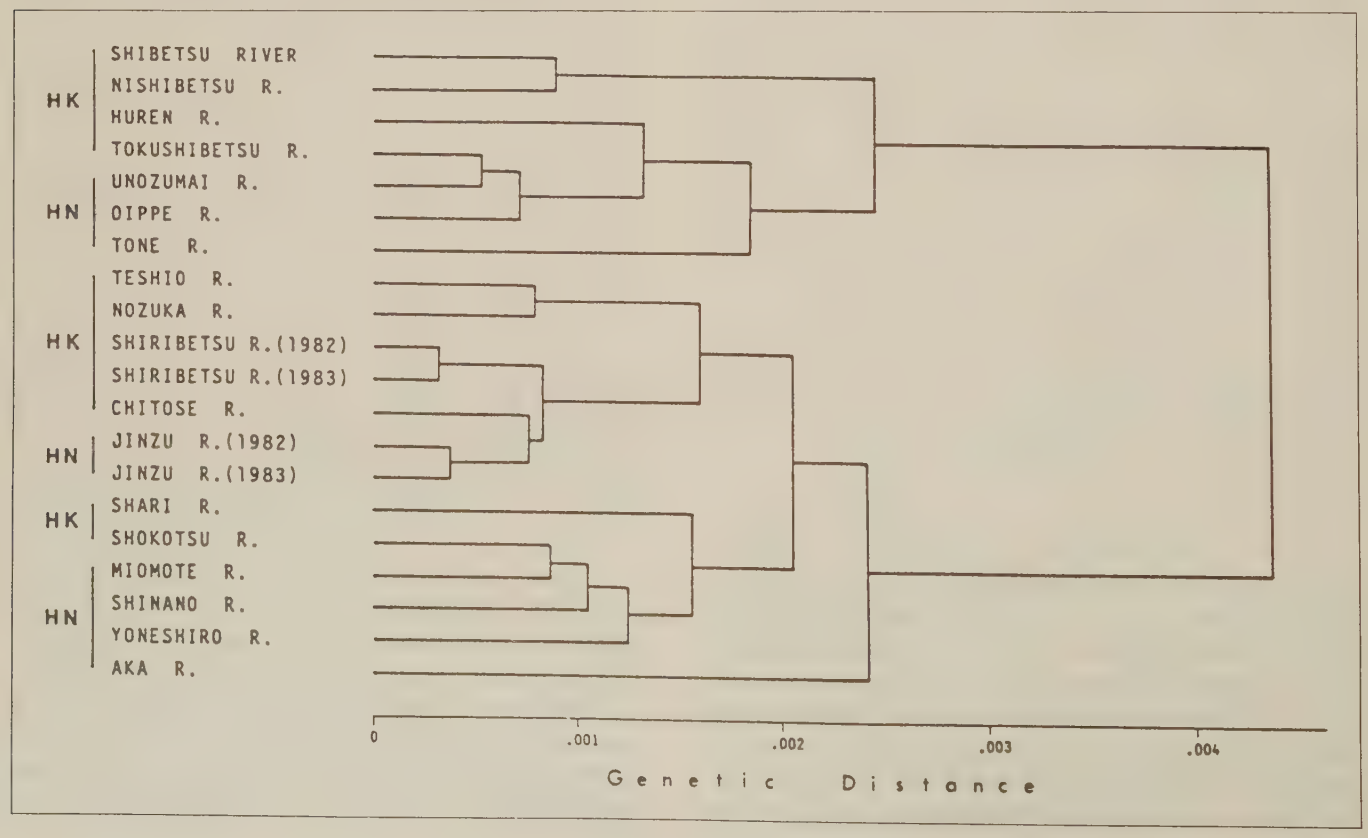
Figure 10
Isopleth diagram of total catch (by weight) and the percentage of total spawns (eggs) for masu salmon, against availability rate by age (in ten-day units), Q, and fishing mortality coefficient, F. Unit of contour numerals are in 1,000's of metric tons (Kato and Hasegawa 1986).

the virgin resources in Figure 10. Values are calculated using the fishing mortality rate and the survival rate during each of the ten-day periods. The fishing mortality coefficient (F) is plotted from 0 to 0.20 from the starting time of the fishery at 80 ten-day units (late December) to 108 ten-day units (end of the life span in early October the following year) showing availability by age in ten-day units (Q). As shown by the black spot in Figure 10, the present statuses of both the reproduction rate of the resource (total spawn) and the fishing mortality coefficient are 10% and 0.111, respectively. It is therefore necessary to cut the intensity of the fishing mortality rate by 36% (0.07/0.111) to decrease the total catch from 3,000 to 2,500 metric tons and ultimately improve the reproduction levels of the masu salmon from 10% to 30% in total spawn. Although masu salmon resources of the Japan Sea have a tendency to decrease owing to over-fishing, they are able to increase during years where there is an expansion of the number of effective spawns and smolt-releases.

Genetic Feature

Genetic variation and population structure of masu salmon river populations were examined using electrophoretic methods to compare enzymatic proteins in samples of fish

Figure 11 (below)
Estimation of genetic distance among twenty populations of masu salmon in Japan. HK: Hokkaido; HN: Honshu (Okazaki 1986).



among twenty populations collected from eighteen rivers. Although no clear structuring of the population on a geographic basis was discovered, a rough division into two groups appeared at the Shiretoko Peninsula (except for several river populations), as shown in Figure 11; (1) the rivers entering the strait of Nemuro and the Pacific Ocean, and (2) the rivers entering the Okhotsk and Japan Seas (Okazaki 1986). Still, the genetic independence of river populations is recognized distinctly. These observations suggest a possible relationship with the instinctive homing nature of masu salmon.

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Nutritional Approach to the Production of Masu Salmon (*Oncorhynchus masou*) Smolt

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ABSTRACT

Little attention has been paid to the possible role of nutrition in the field of salmonid smolt production. At the physiological level, the parr-smolt transformation (smolting) accompanies a marked decline in reserve nutrients in the body and an increase in oxygen consumption. The elevated catabolic status observed during smolting suggests that the nutritional requirements of smolt must be different from those of parr. A 120-day feeding test was conducted to study the effects of dietary proteins and lipid levels on growth and smolting of yearling masu salmon (mean body weight, 16 g). The following four diets were used in this study: HPHL, high protein 42%-high lipid (16%); HPLL, high protein (42%)-low lipid (4%); LPHL, low protein (24%)-high lipid (14%); LPLL, low protein (24%)-low lipid (2%). The growth response to these dietary treatments was as follows: HPHL (final mean body weight, 44.4 g) > HPLL (36.5 g) > LPHL (29.0 g) = LPLL (28.0 g). Only in the high protein diet group, both growth and feed efficiency were distinctly improved by lipid supplementation. In all treatment groups, growth, feed efficiency, and food consumption rate were accelerated with the onset of smolting. The percentages of the smolts obtained were 74.5%, 47.3%, 29.1%, and 16.4% for HPHL, HPLL, LPHL, and LPLL, respectively. The improvement of fish performance seemed to result from enhancement of the digestible energy content in the diet and from elevation in the dietary levels of essential fatty acids. The present results show that nutrition affects growth as well as smolting.

Introduction

Young anadromous salmonids exhibit characteristic morphological, physiological, and behavioral changes during the parr-smolt transformation (smolting) prior to actual seawater entry (Hoar 1976; Folmar and Dickhoff 1980). Methods for controlling and enhancing the smolting have been intensively studied based on physiological data. At present, physiological treatments such as temperature, salinity, and photoperiod regimes are known to influence growth and smolting drastically (Saunder and Henderson 1970; Wagner 1974; Knutsson and Grav 1976; Ewing et al. 1980; Clarke et al. 1981; Sato et al 1986). On the other hand, little attention has been paid to the possible roles of nutrition in smolt production. Unlike physiological and endocrinological treatments, nutritional treatment might indirectly stimulate the transformation.

At the biochemical level, the smolting accompanies a marked decline in reserve nutrients (lipid and glycogen) in the body and an increase in oxygen consumption (Fontaine and Hatey 1950; Saddler et al. 1972; Ota and Yamada 1974; Woo et al. 1978; Sheridan et al. 1985; Sheridan 1986; Virtanen 1987). From a nutritional point of view, the elevated metabolic status of smolting fish seems to be reflected in body composition changes, thus the nutritional requirements of smolt would be different from those of parr. Therefore, we have conducted several nutritional studies to develop a diet formulated for the production of masu salmon (*Oncorhynchus masou*) smolts. Some of the results will be summarized in this report.

Comparison of Body Composition between Smolts and Parr

There has been little information published on the nutritional requirements of smolting masu salmon. We initially examined the differences in body composition between

smolts and parr of masu salmon during March through May (Ogata and Konno 1986). The most striking difference found was in the body lipid content as has been shown in other salmonids. The nonpolar lipid contents in the whole body, liver, and skeletal muscle were clearly lower in the smolts than in the parr, while no difference in the polar lipid contents was detected (Fig. 1). During this same period the trend for nonpolar lipids in the body mirrored that of neutral fat (triacylglycerol) in the blood, where neutral fat levels were always lower in the smolt than in the parr (Fig. 2). In contrast, the smolts showed higher fatty acid levels in the blood than the parr throughout this period (Fig. 3). Thus during smolting, the body's source of energy reserves decreased markedly, and the metabolism of nutrients was intensified, probably owing to the accelerated excretion of certain hormones. Based on these findings, we proposed the idea that supplementation of lipids to the diet of fish undergoing smoltification would lead to an improvement of growth, energy utilization, and the entire smolting process as a consequence.

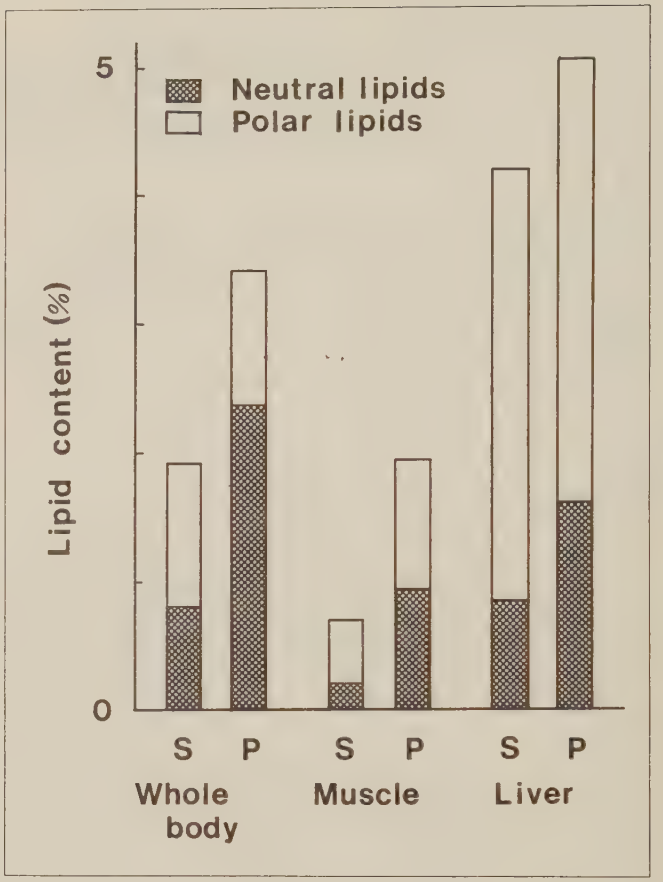

Figure 1
Comparison of lipid contents between the smolts and parr of yearling masu salmon (Ogata and Konno 1986): S = smolts; P = parr.

Effects of Lipid Supplementation to Diet

Two feeding tests were conducted to examine the effects of dietary lipid content on growth and smolting using underyearling and yearling masu salmon. In the first study using underyearlings, four test diets containing 4%, 8%, 12%, and 16% lipid were prepared using a mixture of pollock viscera oil and soybean oil (2:3, v/v) as the lipid source. Vitamin-free casein supplemented with amino acid

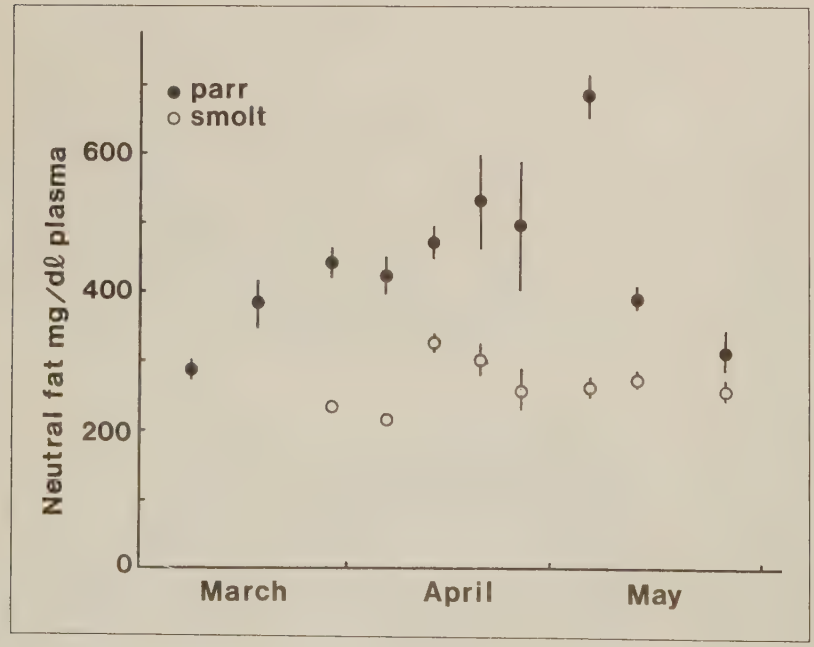

Figure 2
Changes in the neutral fat levels in the plasma of yearling masu salmon from March through May (Ogata and Konno 1986).

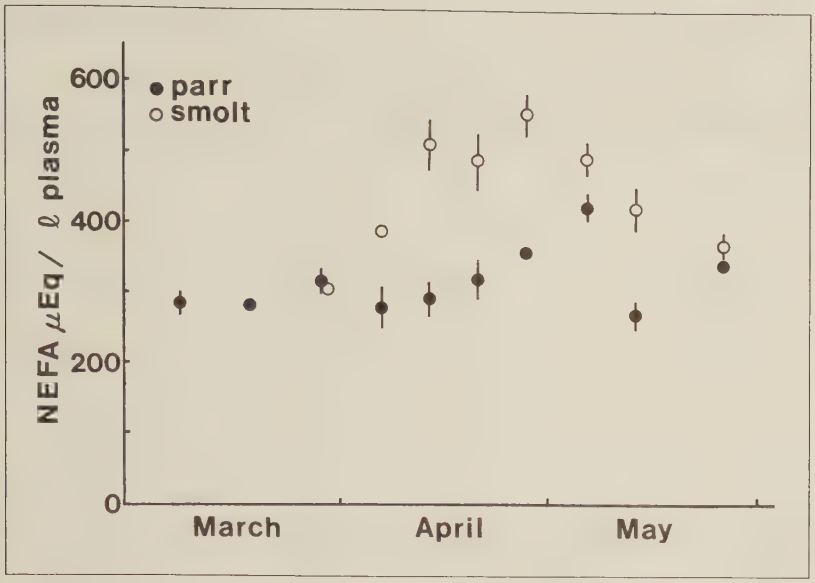

Figure 3
Changes in the fatty acids in the plasma of yearling masu salmon from March through May (Ogata and Konno 1986). NEFA = non-esterified fatty acids.

mixture was the protein source (Ogata et al. 1983), and crude protein levels of all the diets were adjusted to about 40%. Each replicate of 40 fish (mean weight 1.46 g) were stocked in a PVC tank. Each of the four dietary treatments consisted of duplicate tanks where fish were fed each of the four test diets. Well water at 15°C was supplied continuously. Fish were fed by hand to satiation twice daily, 6 days per week for six weeks.

Measured after six weeks, food consumpton rate decreased linearly as the dietary lipid level increased (Table 1). The percent weight gain for the 6 weeks also showed a similar relationship (data not shown). These results indicate that for masu salmon weighing less than 4 g, excessive dietary lipid content depressed food consumption and consequently growth.

In the second study, we examined the effects of dietary lipid and protein levels on performance of the yearling fish undergoing smolting. In this study, white fish meal was used to provide about 41% crude protein in diets No. 1 and 2, and 24% in diets No. 3 and 4 (Table 2). Pollock viscera oil was also supplemented to elevate the dietary lipid contents of No. 1 and 3. Thus four test diets were prepared: high protein (41%)-high lipid (16%), HPHL; high protein (41%)-low lipid (4%), HPLL; low protein (24%)-high lipid (14%), LPHL; low protein (24%)-low lipid (2%), LPLL. Fifty-five fish (16 g mean body weight) were distributed randomly into four PVC tanks, which were continuously supplied with well water at 15°C. Each tank contained one treatment. The fish were fed their respective test diets by hand to satiation twice daily, 6 days per week for 120 days (January through May). Smoltification was determined using the methods of Kubo (1980).

The growth response to these dietary treatments was as follows: HPHL, final mean body weight, 44.4 g; HPLL,

Table 1
Performance of underyearling masu salmon after six weeks of being fed the experimental diet. Values are the average of two duplicate tanks containing forty fish each.

Diet	Protein lipid (%)	Average body weight (g)		Weight gain (%)	Daily food consumption (%)
		Initial	Final		
1	40-4	1.46	4.06	178	2.00
2	40-8	1.45	3.84	165	1.84
3	40-12	1.45	3.76	159	1.80
4	40-16	1.46	3.58	145	1.75

Table 2
Formulations of experimental diets (% by weight).

Diet composition	Diet no.			
	1	2	3	4
White fish meal	64.64	64.64	35.91	35.91
Brewer's yeast	5.00	5.00	2.78	2.78
Torula yeast	3.00	3.00	1.67	1.67
Pollock viscera oil	12.00	0	12.00	0
Dextrin	5.00	18.00	18.00	30.00
Cellulose flour	2.86	1.86	21.14	21.14
Mineral mix	2.50	2.50	3.00	3.00
Vitamin mix	2.00	2.00	2.50	2.50
CMC[a]	3.00	3.00	3.00	3.00
Crude protein	40.9	40.9	24.3	24.1
Crude fat	15.6	3.9	14.2	2.2

[a]Carboxymethyl cellulose sodium salt.

Table 3

Performance of yearling masu salmon after 120 days of feeding the experimental diets.[a] HPHL = high protein, high lipid; HPLL = high protein, low lipid; LPHL = low protein, high lipid; LPLL = low protein, low lipid.

	HPHL (1)	HPLL (2)	LPHL (3)	LPLL (4)
Average body weight				
Initial	15.48	15.83	16.69	16.06
Final	44.41	36.47	29.04	28.02
Weight gain (%)	187	130	74	74
Feed efficiency	0.60	0.48	0.31	0.28
Daily food consumption (%)	1.34	1.29	1.27	2.33
Number of smolt obtained[b]	41/55	26/55	16/55	9/55

[a]Each tank contained 55 fish.
[b]Number of smolt obtained relative to initial number of fish. Differences in numbers of the smolt obtained were statistically significant (χ^2-test, $P<0.01$) among the dietary treatments (Ogata and Konno 1986).

36.4 g; LPHL, 29.0 g; LPLL, 28.0 g (Table 3; Fig. 4). As seen in the growth response, the best feed efficiency was achieved by using the HPHL diet. Thus only when fish were given a high protein diet, did both growth response and feed efficiency clearly improve. Lipid supplementation to the high protein diet distinctly improved the rate of smolt production, and the percentage of smolts obtained were 74.5%, 47.3%, 29.1%, and 16.4% for HPHL, HPLL, LPHL, and LPLL, respectively.

These results show that nutrition affects growth as well as smolting and suggest that lipid supplementation to a diet is a valuable method for promoting smolt production. However, Plotnikoff et al. (1983, 1984) reported that dietary treatments failed to show any effects on the osmoregulatory abilities of chinook salmon before seawater entry, even though growth, food, protein, and energy utilization were improved by feeding a high lipid diet. More studies on the effects of dietary composition, not only on the apparent rate but also on the completeness of smolting, are needed to elucidate the relationship between nutrition and smolting.

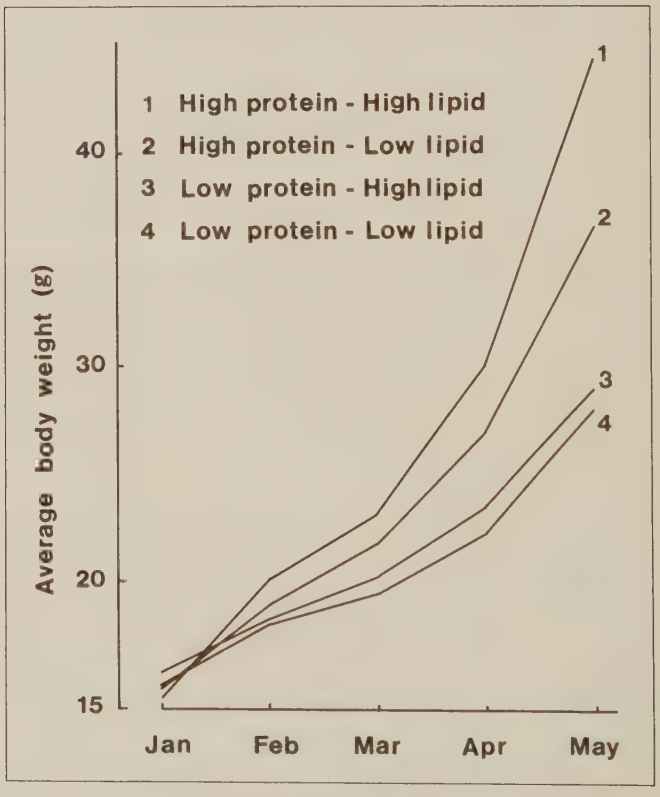

Figure 4
Changes in mean body weights of the fish during the test period.

Conclusion

In the present report, we found differences in the body composition of underyearling and yearling masu salmon, and their response to a diet fortified with lipid. Supplementation of lipid over 4% in dietary lipid level depressed the food consumption and growth of the former fish. By contrast, when they were given a diet enriched with lipid slightly prior to the onset of smolting, growth, feed efficiency, and smolting rate were distinctly improved. In a later study, these improvements were shown to result not only from the enhancement of the digestible energy content in the diet but also from the elevation in the dietary levels of essential fatty acids (Ogata and Murai 1989).

Acknowledgments

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An Approach to the Efficient Enhancement of Masu Salmon through the Release of Juveniles into Streams

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ABSTRACT

The masu salmon (*Oncorhynchus masou*) is one of the most important fishery resources in northern Japan along with chum (*O. keta*) and pink (*O. gorbuscha*) salmon. In the last decade, the chum salmon resource has increased dramatically, while the overall levels of masu salmon have decreased. This is due to several complex factors involving the biological characteristics of masu salmon and human activities. Considering the present number of facilities and the constraints on the annual budget, it is difficult to quantitatively increase smolt production using present methods. In this paper we discuss the release of masu fry upstream in the spring, a new approach which, when used in combination with other methods, can help increase smolt production without requiring additional investments in new man-made hatcheries. In our study, efficiency of smoltification was confirmed by measuring the resulting fingerlings in the fall. It is suggested that the level of masu salmon returning to the study area (Mena River), which is the highest in over fifty years, may be a result of this new technology. In addition, we review similar research on fingerlings released just prior to the wintering period. The river's maximum hatchery potential is realized when fry release is properly timed at locations with existing smolt development habitat.

Introduction

Chum salmon (*Oncorhynchus keta*) resources have increased dramatically in the last decade, marking 100 years since artificial salmon propagation was earnestly introduced in Hokkaido. Conversely, masu salmon (*O. masou*) levels seem to have decreased. Considered one of the important coastal fishery resources in northern Japan, an increase in the masu stocks would have significant economic benefits. In this report, we summarize the situations and problems around masu salmon propagation, especially in Hokkaido. The effectiveness of fry release to the uppermost positions of tributaries and fingerling release before wintering are also discussed. Both are newly introduced concepts recently investigated under the Marine Ranching Program.

History of the Masu Salmon Fishery

Accurate landing statistics of masu salmon in coastal and offshore waters alone are not available. However, changes have been observed in masu salmon catches in the rivers where artificial propagation has been carried out in Hokkaido (Fig. 1). For comparison the level of chum salmon harvested in the coastal areas and rivers in Hokkaido is also presented. In comparison with chum salmon increases, masu salmon catches are stagnant. This may be the result of several complex factors, one being the masu salmon's intrinsic biological characteristics and another the active role of humans, which we discuss later in this text.

Masu Salmon Biological Characteristics

Life Cycle—The life cycle of the masu salmon is schematically shown in Figure 2. Masu salmon spawn at the uppermost positions of tributaries in the fall, emerge as fry from the redds, disperse during the spring flood, and begin growth. After spending one year in freshwater, most reach 10–11 cm in fork length, undergo smoltification, and migrate downward to the sea. The remainder either spend an extra year in freshwater prior to migrating, or, as in the case of precocious male fish, spend their whole life in freshwater (Tanaka 1965; Machidori and Kato 1984). The masu salmon which migrate to the sea spend one year

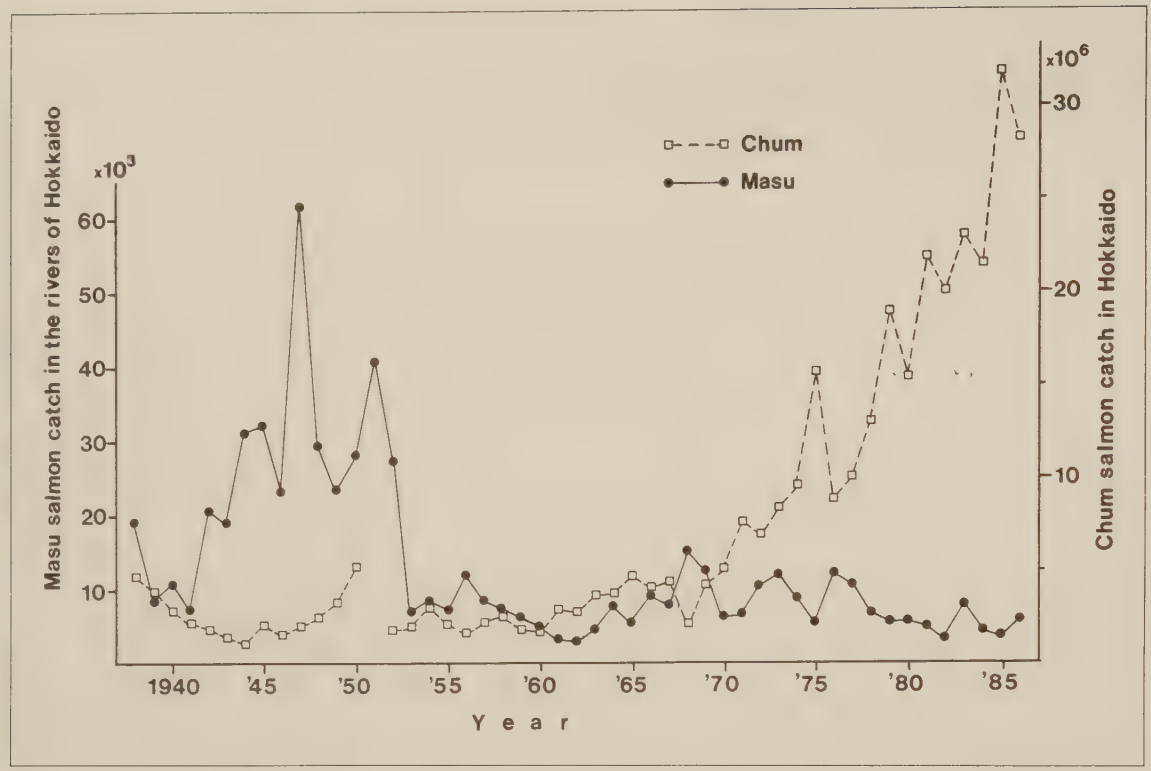

Figure 1

Annual change in masu salmon catch in rivers of Hokkaido and in chum salmon catch in rivers and coastal areas of Hokkaido. Chum salmon data prior to 1951 from Mihara et al. 1951. Other information from Hokkaido Salmon Hatchery, Activity Department (unpubl. data).

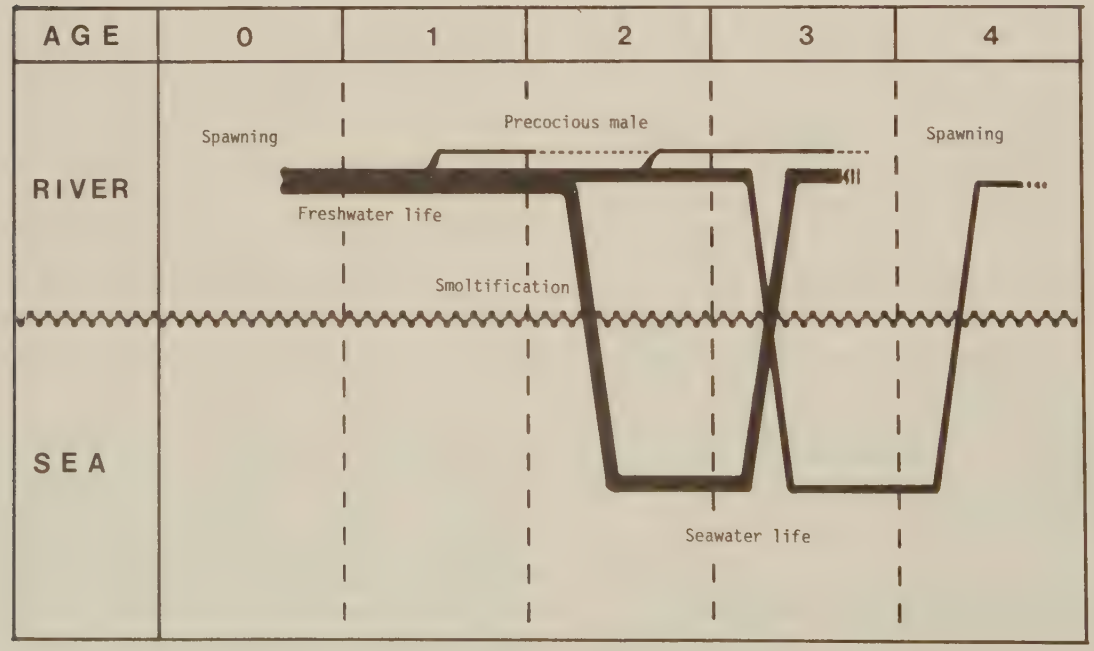

Figure 2

Life cycle of masu salmon in northern Japan.

there, then migrate back to the coastal area around their natal river's mouths. Entering the rivers soon after returning, they spend the summer in the deepest depths of the rivers, then continue upstream to their spawning area in the fall.

Enhancement—The problems facing masu salmon propagation range widely. Owing to low food productivity in rivers where masu salmon spend at least one year before migrating to the sea, the standing crop of fish is easily depressed. There are two biological factors which affect the standing crop. One is regulation of growth and the other is control of total biomass; neither of which seems capable of bringing about either an explosive increase or a deadly decrease in the amount of masu salmon stock. This appears to be a survival strategy for masu salmon that depend heavily on rivers with low food productivity.

Human activities such as the linearizing of stream courses and the covering of stream banks with concrete reduce the amount of suitable fish habitat. Furthermore, dams and other facilities in the rivers prevent upstream migration for masu salmon during spawning and also the dispersion of fry.

The artificial propagation of this species poses various problems. Masu salmon are caught at a fish weir installed near the river mouth in the spring to avoid illegal catches upstream. For this reason, they must be held in ponds for more than two months until fall, and consequently, the number of deaths during this period is rather high. Recent improvements in the holding technique have reduced the mortality of masu salmon during this period (Hiroi 1988). The annual release of artificially produced masu fry is conducted every year in a manner similar to that used for chum fry by releasing large numbers from only a few points in the middle reaches of the rivers. Furthermore, angling for the resulting fingerlings by sport fishermen is also thought to be extremely harmful on the propagation of masu salmon.

Comparing our ability to use female adult masu with our ability to use female chum salmon for extraction, it is clear that the average number of masu eggs collected in Hokkaido is lower than that of chum salmon because of loss during the holding period (Fig. 3)—a great loss of eggs that are necessary for the production of seed. The highest average number of eggs per fish was obtained from fish in the Shiribetsu River.[1] Adult masu salmon are captured in the fall in the Mena River, a branch of the Shiribetsu. Because capture occurs closer to their spawning period, the holding time before the eggs are taken is shortened, thus reducing stress to the fish during maturation.

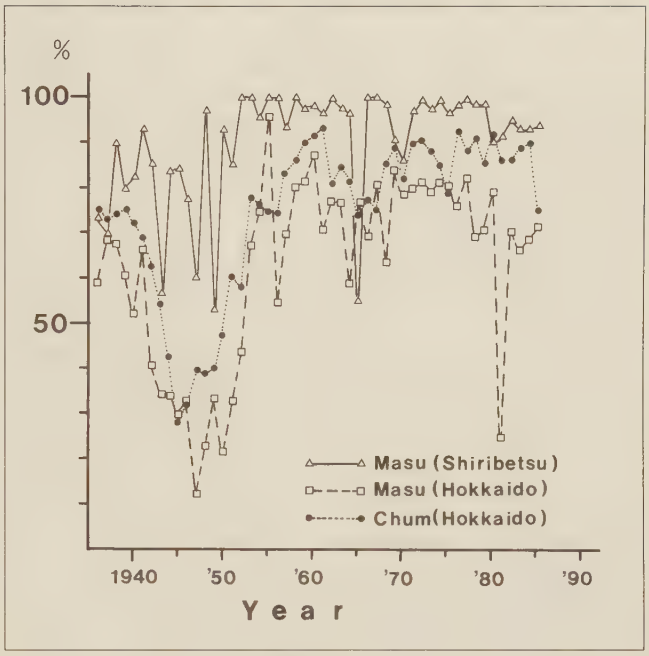

Figure 3
Annual change of rate of availability of female masu salmon which were caught for artificial propagation (Hokkaido Salmon Hatchery, Activity Dept., unpubl. data.)

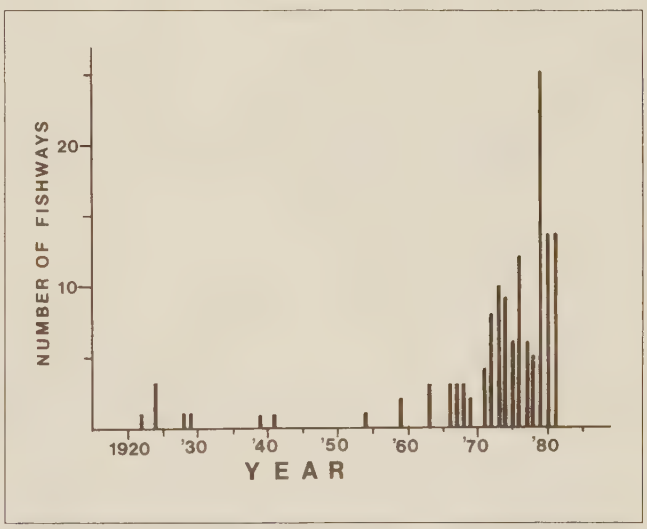

Figure 4
Change in the number of fishways constructed annually in Hokkaido. (Data from Fisheries Dept., Hokkaido government.)

Figure 4 shows the annual change in the number of fishways installed next to dams or other facilities from 1920 to 1981. Although the number of fishways has increased

[1] "Number of eggs per fish" refers to the highest average rate of egg stripping efficiency to total female masu salmon caught at the weir, not fecundity of fish.

$$\text{Yearly rate} = \frac{\text{No. of female salmon caught and stripped}}{\text{No. of female salmon caught at the weir}} \times 100\%.$$

$$\text{Average rate} = \frac{\Sigma \text{ yearly rate}}{\text{No. years.}}$$

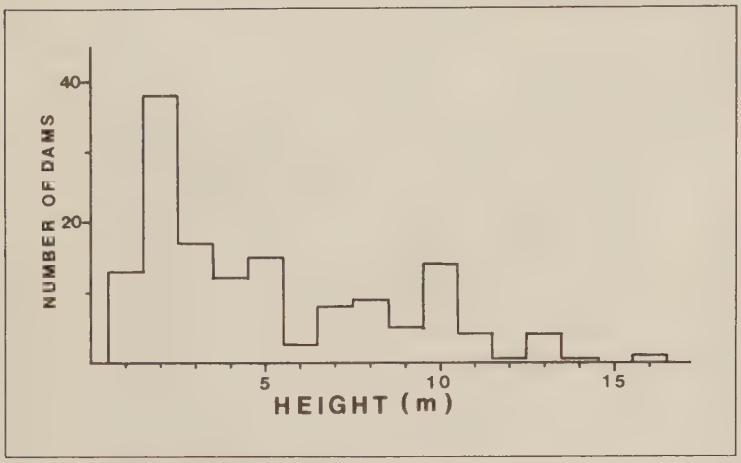

Figure 5
Distribution of the height of dams where fishways are installed in Hokkaido. (Data from Hokkaido government.)

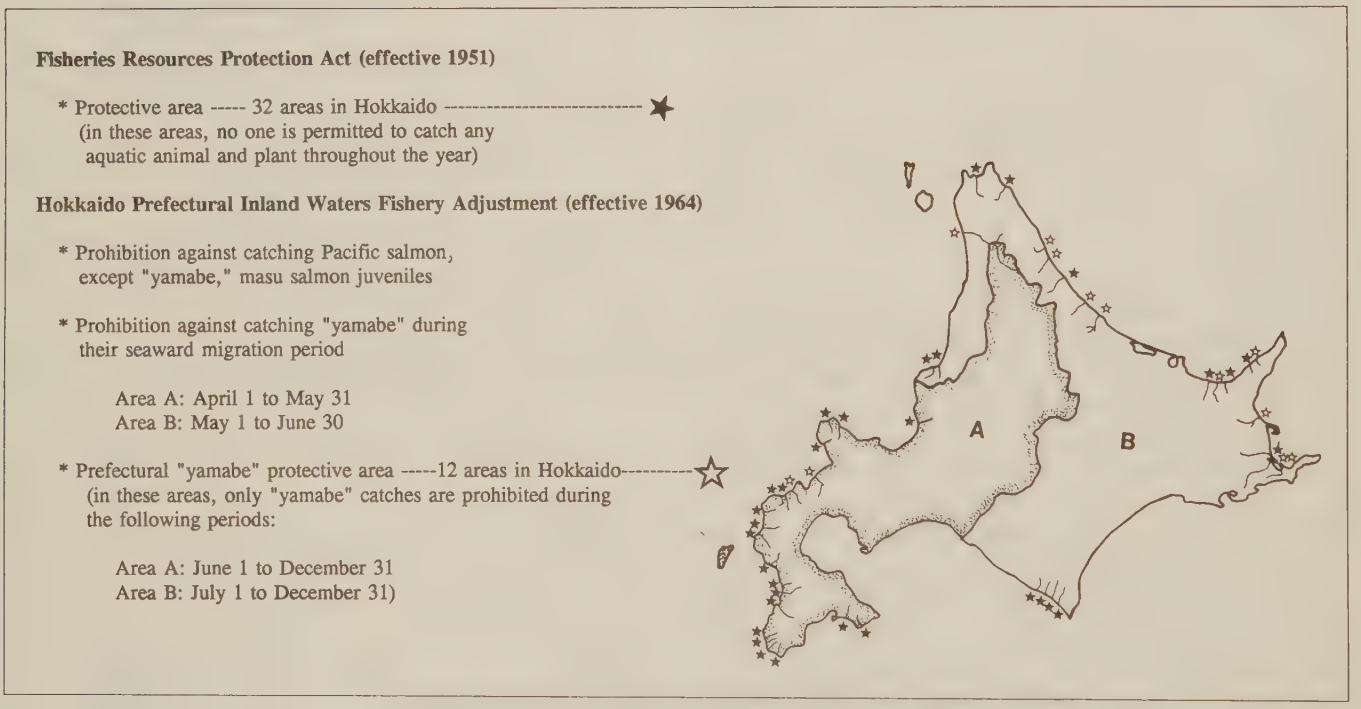
Fisheries Resources Protection Act (effective 1951)
* Protective area ----- 32 areas in Hokkaido ----------
(in these areas, no one is permitted to catch any aquatic animal and plant throughout the year)
Hokkaido Prefectural Inland Waters Fishery Adjustment (effective 1964)
* Prohibition against catching Pacific salmon, except "yamabe," masu salmon juveniles
* Prohibition against catching "yamabe" during their seaward migration period
Area A: April 1 to May 31
Area B: May 1 to June 30
* Prefectural "yamabe" protective area -----12 areas in Hokkaido---------
(in these areas, only "yamabe" catches are prohibited during the following periods:
Area A: June 1 to December 31
Area B: July 1 to December 31)
A
B

Figure 6
Regulations governing masu salmon catch in freshwater, in Hokkaido.

in recent years, most of them have been installed next to dams which have a height of less than 5 meters (Fig. 5), and many dams are still left without any route for fish to migrate upstream.

The salmon fishing regulations for the inland waters of Hokkaido are complex and strict (Fig. 6). Thirty-two areas are designated as protected areas by the Fisheries Resource Protection Act of 1951. In these areas, catching or collecting any kind of aquatic animal or plant is prohibited throughout the year. Hokkaido Prefectural Inland Waters Fishery Adjustment additionally forbids all Pacific salmon fishing except for "yamabe," the fingerling of masu salmon. In the case of yamabe, this adjustment forbids fishing during the two-month period of their seaward migration which varies geographically. Finally in 12 prefectural yamabe protective areas, the adjustment extends this ban from the end of the migration period through the last day of the year. Therefore it is possible to angle masu salmon in many locations, even in the 12 regulated areas, but only during the open periods.

Present Fishery Techniques

Release of Juvenile Fish

Although smolt production and release is the main technique used to enhance this resource in the Marine Ranching Program (Mayama 1991), it is difficult to enlarge smolt

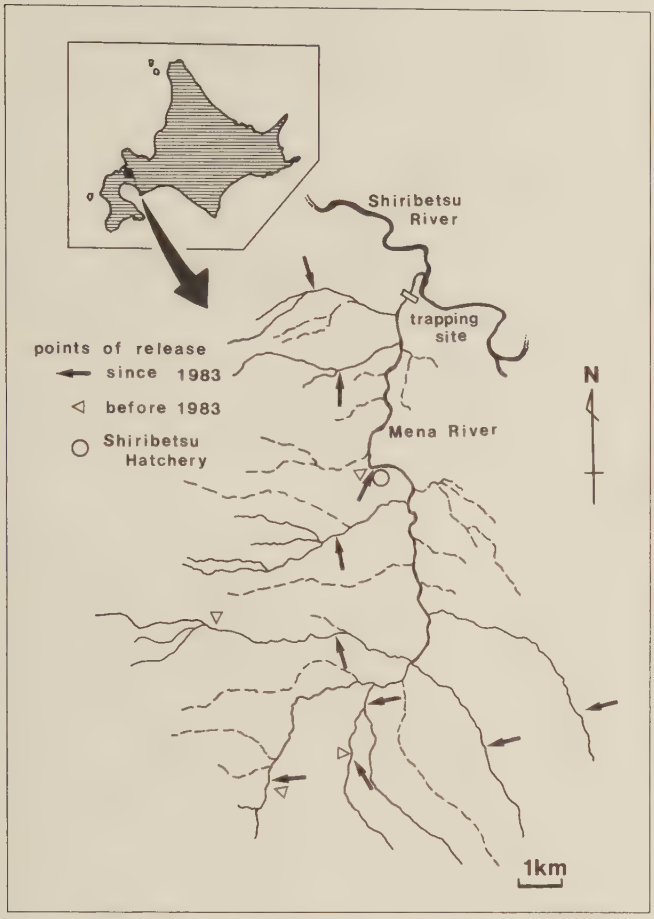

Figure 7
Location of the Mena River. Masu salmon fry had been released from the points indicated by triangles before 1983 and released from the points indicated by arrows with controlled number of fish from tributaries since 1983.

Table 1
Data on released fry and their return during the period of the experiments. WL is the distance from the center of the nucleus to the edge of the first freshwater annulus of the scale and is indicated in mean value.

Brood year	Date of release	Average fork length at release (cm)	Number of released fry (thousands)	Average fork length in the summer (cm)
A) Fry release				
1980	late May 1981	4.05	1,221	7.7
1981	late May 1982	3.5	522	9.6
1982	24–25 May 1983	4.62	400	9.0
1983	28–29 May 1984	4.85	800	8.5
1984	21–22 May 1986	5.26	630	8.5
1985	20–21 May 1986	4.73	600	—
1986	26–27 May 1987	4.55	627	—

Brood year	Year of return	Catch in the river	WL of 3 year female fish[a] (mm)	WL of 3 year male fish[a] (mm)
B) Adult return[b]				
1976	1979	1,200	0.378	0.390
1977	1980	1,578	0.365	0.336
1978	1981	660	0.423	0.442
1979	1982	679	0.411	0.426
1980	1983	931	0.407	0.408
1981	1984	537	0.436	0.426
1982	1985	425	0.450	0.468
1983	1986	1,268	0.428	0.447

[a] Fish measured were predominantly of native origin.
[b] From database used by Ohkuma (1988).

production in the hatchery with the present facilities and annual budget. Therefore, it is hoped that this method can be used in combination with other effective strategies. Accordingly, we have experimented with the following two approaches stated previously. One is to release fry in the uppermost positions of as many tributaries as possible, and the other is to release fingerlings before the wintering period.

Fry Release and Sampling Method

The fry release experiments were held in the Mena River, a branch of the Shiribetsu River (Fig. 7). Prior to 1983, large numbers of fry (from about 300,000 to 1,000,000 per site) were released from only a few locations (Shiribetsu Hatchery and several other sites upriver). Beginning in 1983, smaller numbers of fry (usually 30,000 to 50,000 per site) were released at each of a larger number of release points in order to improve fry dispersion and to maximize the productivity of the river system.

The information about those released fry is shown in Table 1A. These averages are obtained from a single observation point in the middle reaches. The 1981 released group (the 1980-brood-year group) grew very slowly, and consequently, the average fork length of the group did not reach over 8 cm during the summer. Many masu salmon adults migrated upstream over the fish weir in the fall of 1981. Because swimming fry, assumed to be wild fish, were confirmed in most of the tributaries in the spring of 1982, we released masu fry only to the lower areas of the river from the hatchery in that year. Therefore, it is highly probable that the value of 9.6 cm in average fork length better represents the fish which originated by natural spawning. Results obtained after the 1983 release represents that of released fish growth after summer, and consequently the average fork length reached over 9 cm before wintering, showing improvement over released fish measured in 1981.

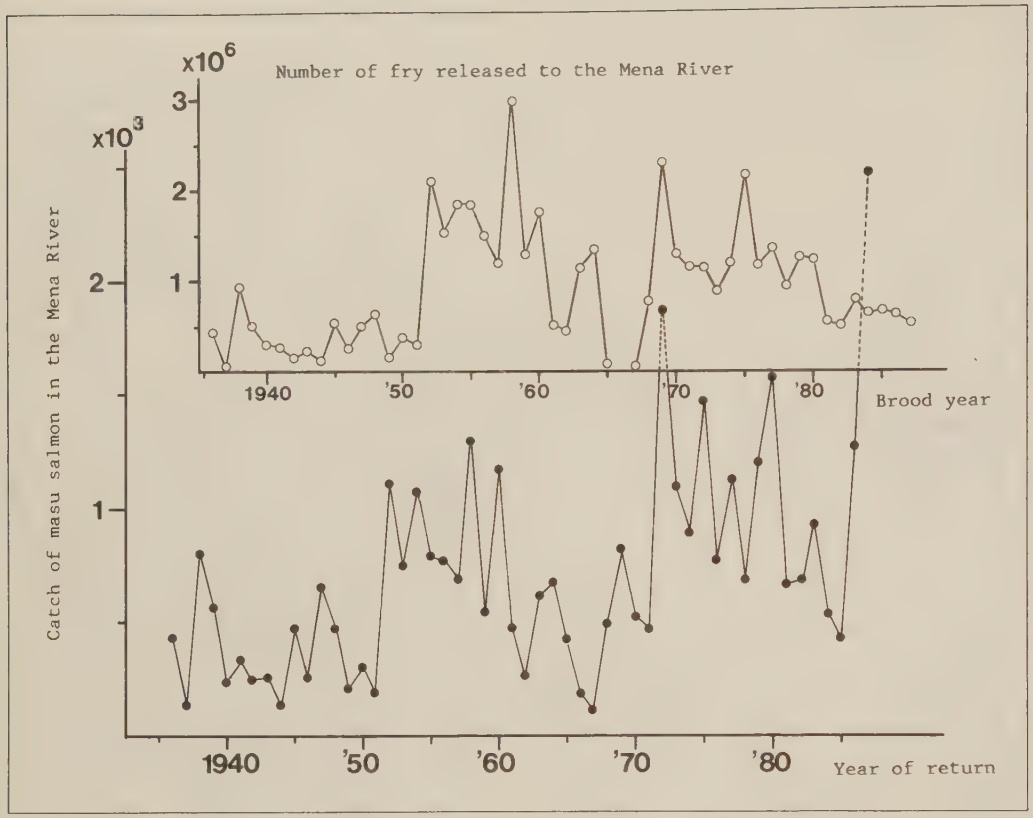

Figure 8
Changes in the number of released fry (upper) and returned adult masu salmon (lower) in the Mena River, a branch of the Shiribetsu River. No fry were released officially in 1966. (Hokkaido Salmon Hatchery, Activity Dept., unpubl. data.)

Adult return information is shown in Table 1B (Ohkuma 1988). The scale radius, or distance from the center of the nucleus to the edge of the freshwater annulus, was measured in order to estimate growth during their freshwater life. It shows that the radius of the 1982 brood year group was comparatively large.

In order to confirm the effectiveness of multiple point, upstream fry released with respect to dispersion, the relationship between the number of released masu fry and returning adults in the Mena River was investigated (Hokkaido Salmon Hatchery, unpubl. data; Fig. 8). Although the number of released fry had been small until around 1950, it reached over 1½ million at various times after that. As for the adult return, it had increased by over 1,000 in 1952, but it decreased again in the late 1960s. In 1970 the Mena River was designated as a yamabe protective area, and coincidentally, the number of returning masu salmon increased to more than 1,000 for several years afterwards. Unexplainably, it again fell in the early 1980s. Since 1983, when the dispersing method took place in earnest, the adult return increased gradually in 1986 and 1987 after reaching a bottom in 1985, even though the number of released fry were maintained at low levels. In 1987, more than 2,000 returning fish marked the highest level of returns in 50 years.

In the artificial masu salmon propagation program being carried out in Hokkaido at the present time, fry are released into middle reaches of rivers at a size of 4 to 5 cm in fork length. Because of poor swimming ability of these small fry, it is difficult for them to disperse upstream; therefore, food productivity in the areas upstream from the release locations cannot be utilized effectively. On the other hand, the new method we have examined at this time resolved this problem by releasing fry to the uppermost tributary positions, the numbers of which depend on the stream's carrying capacity. This method is superior to the presently used release method because it uses the whole stream for smolt production. Although it requires more effort at the time of release, we believe it results in improved smolt production and adult returns.

Fingerling Release

The release of fingerlings took place in the fall before wintering in the Mena and Shubuto Rivers, in 1982 and 1985, respectively (Mayama et al. 1988; Fig. 9). There is a large dam for production of electric power in the Shiribetsu River. Because the area upstream from the dam cannot be utilized for salmon reproduction or propagaton, the artificial propagation has been taking place in the Mena River only. The masu salmon of this river spend the summer in the deepest depths of the Shiribetsu. In the fall they enter the Mena River to spawn and, as discussed earlier, are caught with almost fully matured gonads. Thus, a high

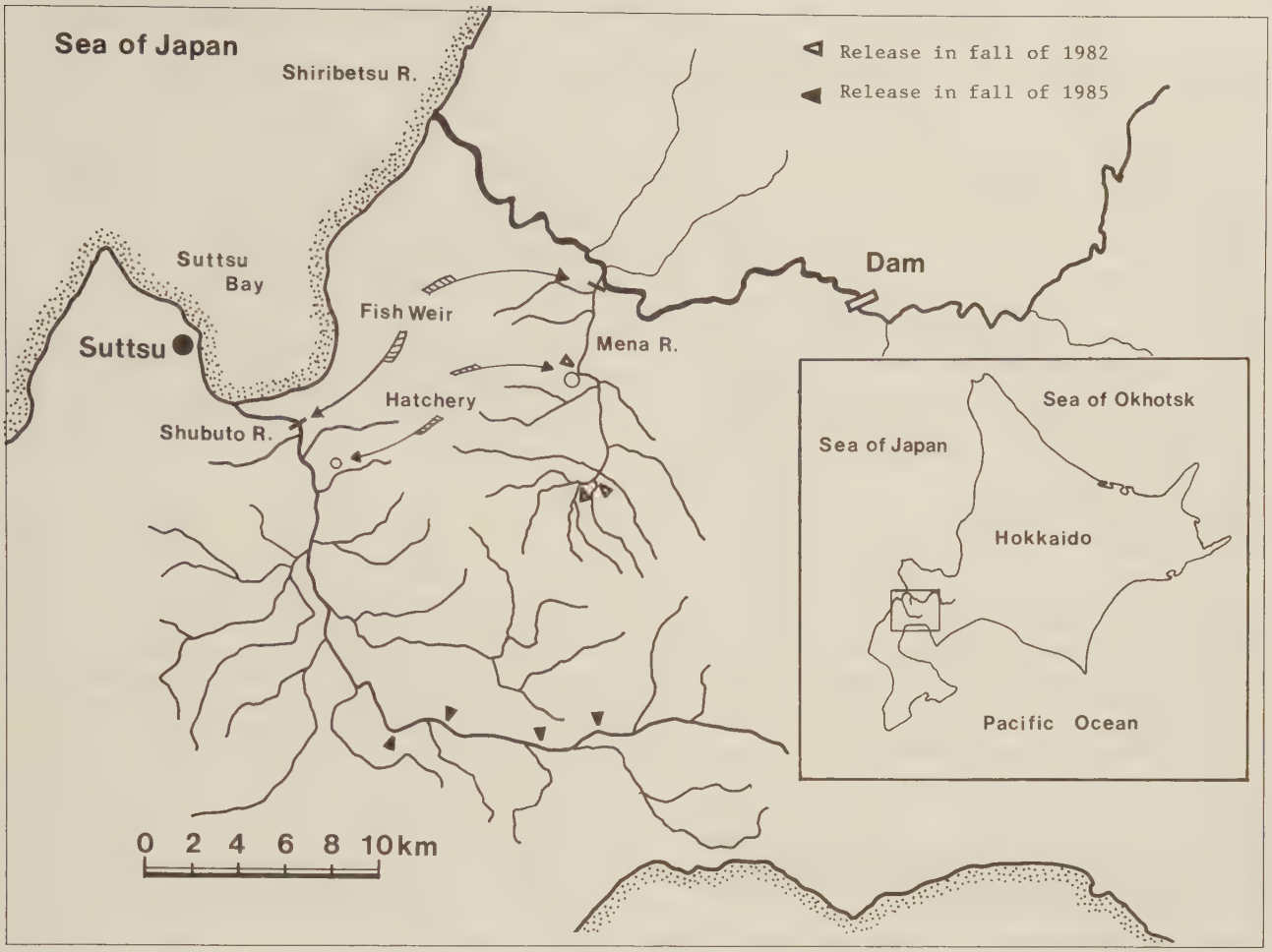

Figure 9

Location of the Shubuto River and the Mena River where masu salmon fingerlings were experimentally released before wintering.

rate of utilization for egg extraction is maintained (Fig. 3). Fingerlings were released from three points in the Mena River and from four points in the Shubuto River. The locations for fingerling release were chosen using the following criteria: a slow rate of river flow, presence of a large pool nearby, and ease of fingerling dispersion from the area. After wintering in a suitable space, released fingerlings migrate to the sea the next spring. When they migrate back to the Suttsu coast in the spring two years after release, they are caught in a stationary net located in the nearby coastal area.

Table 2 shows information on fingerling release and their return as adults. In November 1982, 53,700 year-old fingerlings of the 1981 brood year (9.11 cm average fork length) were released into the Mena River marked by clipped right ventral fins. In the same way, 135,000 fish of 1984-brood-year fingerlings (9.74 cm average fork length) were released to the Shubuto River in 1985, with clipped adipose fins. The released fish dispersed within a short period and most of them were thought to have migrated to the sea as smolt the following spring.

Only 0.1% of the released fingerlings, 35 fish, returned to the coastal area from the 1981-brood-year group. However, the catch of the 1984-brood-year group released in Shubuto reached 1,183 fish, or about 0.9%, which is almost 10 times the level of the 1981 release. While the size of the river and coastal catches of the Mena River stock were almost the same in 1983, the catch at the Shubuto River in 1987 was much lower than the coastal catch. One explanation for this is that most of the fish returning to the Shubuto River may have already gone upstream through the trapping site before the weir was installed in August.

These contrasting results between the return rates of the two release groups were supposed to be due mainly to the difference in the size of each fingerling group at the time of release. It was estimated that the percentage of the fish which could reach the size of 11–12 cm (assumed smallest

Table 2

Data of released fingerling and their return. The return rate of the 1984 brood year group was almost ten time as high as the rate of the 1982 brood year group (Mayama et al. 1988).

Origin	Brood year	Released to	Date	Number of fingerlings	Fork length at release (cm)	Body weight at release (g)	Clipped fin	Rate of matured male fish (%)
Fingerling release								
Mena R.	1981	Mena R.	4–5 Nov. 1982	53,700	9.11	9.36	right ventral	0.7
Mena R.	1984	Shubuto R.	30 Oct.–2 Nov. 1985	135,000	9.74	10.70	adipose	9.5

Brood year	River and year, planted	Year of return	Number of catch along the coast	Number of catch in the river	Number of total catch	Fork length and body weight of captured marked fish		
							FL (cm)	BW (g)
Adult return								
1981	Mena R., 1982	1984	53	42	95	female	57.68	2,460
						male	53.08	1,550
1984	Shubuto R., 1985	1987	1,183	131[a]	1,314	female	56.68	2,320
						male	52.18	1,550

[a]Most of masu salmon returning to the Shubuto River had already gone upstream and the rest of them were thought to have been captured at the fish weir in the river.

size for smoltification) was low in the 1981-brood-year group, while the number of fish reaching that size in the 1984-brood-year group was large.

During the winter season, the standing crop of food organisms (predominated by the order Diptera) reaches its greatest level (Atoda and Imada 1972a, 1972b), and the feeding activity of masu fingerlings is lowest (Mayama et al. 1988). The amount of drifting organisms per unit volume is kept at high levels throughout the winter. Though the discharge of the river increases during the following spring rise period, it is thought that there is still sufficient capacity to assure the growth of fish remaining in the river even after adding fingerlings there before wintering (Mayama et al. 1988).

It is also well known that crude fat content is closely related to feeding activity and metabolism (Ota and Yamada 1974). The change in the crude fat content of masu salmon fingerlings proves that there is a reduction of feeding activity in the winter and an increase in feeding activity in early spring (Nomura 1984; Fig. 10).

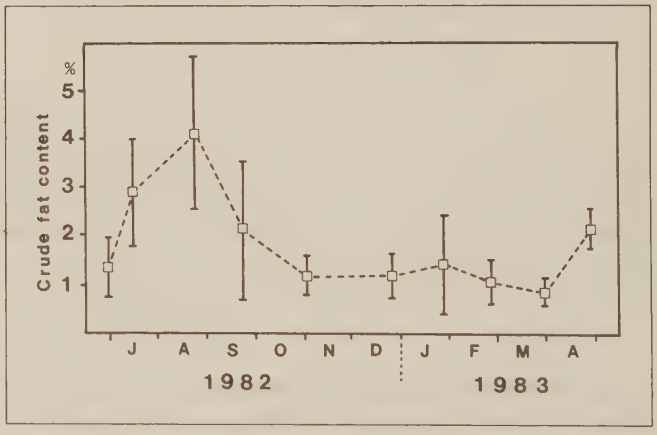

Figure 10

Seasonal changes of crude fat content of masu salmon juvenile in the Mena River. Bars indicate the range of standard deviation (Nomura 1984). J = July; A = August; S = Sept., etc.

Conclusion

Although smolt release is said to be the most effective method of enhancing masu salmon resources, it is difficult to increase the number of smolt because of the high expense and large facilities needed for rearing. On the other hand, a fry release program does not impose higher expenses, although the survival rate and growth of juveniles afterwards closely depends on the conditions at the time of release. Consequently, this method of enhancement may sometimes lead to smaller numbers of smolt migrating to the sea. The effectiveness of fingerling releases before the wintering period has performance characteristics somewhere between fry and smolt release methods, and is effective in the rivers where the circumstances become worse in the summer season. Thus, by combining fry and fingerling release methods with the smolt release program, it

may be possible to efficiently and economically increase masu salmon resources.

Acknowledgments

We would like to thank H. Mayama of the Hokkaido Salmon Hatchery for his valuable guidance and helpful cooperation throughout the experiments. We are also grateful to K. Matsumura and H. Honma of the Hokkaido Salmon Hatchery; T. Wajima of the Suttsu Fisheries Cooperative for their helpful assistance; and S.C. Hasbrouck of Asahi Culture Center, Sapporo, for his assistance in the English translation.

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Present Status and Future of the Marine Ranching Program

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ABSTRACT

This paper reviews several major fisheries research programs in Japan leading up to and including the Marine Ranching Program. Conducted during the 1980s, the Marine Ranching Program integrates the research capabilities of thirty-six academic, governmental, and private institutions in a three-phase program leading to the initiation of "Multiple-Resource Cultivation Systems." These systems take a comprehensive approach to marine aquaculture by combining biological, physical, and engineering sciences to improve entire environments within the ocean ecosystem to create, in turn, more productive and more manageable aquaculture areas. The resulting technology optimizes ecosystem components such as water flow and natural vegetation to support symbiotic relationships between plants, animals, and the ocean and thus to lead to the creation of areas for natural propagation of important commercial species. Results of this program will support future projects including "the Coastal Fishery Ground Development Program" and the "Marin-novation Plan."

Introduction

Remarkable progress has been made in the area of technical developments for the increase of important coastal fisheries resources during the past two decades. Projects promoted by the Ministry of Agriculture, Forestry, and Fisheries (MAFF) have played a vital role in the realization of cultivated fisheries in Japanese coastal and offshore areas under the current policy which has established a 200-mile fishery zone.

Following the dawn of the ocean development age, the Council on Marine Science and Technology was organized. In 1963 it produced a historically monumental report on a plan for scientific and technological goals for the development of ocean resources. This specific report, called "The First Action Program," identified the areas that should be promoted primarily by the government. Among those projects, the cultivation of ocean fisheries was naturally listed as one of the most important topics.

The Shallow Sea Special Program (in Japanese, "Senkai Betsuwaku Kenkyu" or "Comprehensive Research on the Development of the Fishing Grounds for the Aquaculture and Propagation in the Shallow-Sea Areas") was designed and carried out from 1970 to 1974. From 1977 to 1981, partly based upon the Important Species Large-Scale Aquaculture Experimental Program done by contract with the Fisheries Agency, MAFF, The Special Salmon Project (or "Comprehensive Research on the Development of the Large-Scale Cultivation Technologies for Anadromous Salmon and Trout") was conducted, expanding the results of the previous effort.

In response to the establishment of the 200-mile sovereignty zone policy on fisheries resources in 1977, we started to redevelop our coastal and offshore fisheries by focusing on new types of technology. Thus, the "Marine Ranching Program" was developed. Initiated in 1980, the nine-year-long project was designed to enhance the cultivated, or nonconventional fisheries of important species in the coastal and offshore areas of our nation. This program is recognized as the technical foundation for the realization of cultivated oceanic fisheries into the 21st century. The common but unofficial title of "The Marine Ranching Program" has become a very popular nickname among fishery-related communities; however, the program is officially called "Comprehensive Research on the Development of the Domestication System for the Coastal Fisheries Resources." The word domestication is used to express the goal of creating more manageable, important fisheries

resources in the coastal and offshore areas and of promoting their cultivation like that of domestic animals on land.

The following discussion outlines our efforts, progress, results, and prospects for the future in these three programs.

Shallow Sea Special Program: 1970–74

This program focused on the control of diminutive factors on fish, shellfish, and seaweed throughout their life cycle and on the control of important environmental conditions. It was an epoch-making program because the results of the research had to be demonstrated by in-situ experimentation. It should be recognized as the first program in which researchers in the fields of marine biology and marine engineering worked together.

The target species were scallop (*Patinopecten yessoensis*) along the Okhotsk coast (northeast side of Hokkaido), abalone (*Haliotis discus hannai*) along the San-riku (the northeast Pacific coast of the northern Main Island), red seabream (*Pagrus major*) in the Seto Inland Sea (semi-closed sea in western Japan), and Kuruma prawn (*Penaeus japonica*) in Yamaguchi and Oita Prefectures (western edge of Seto Inland Sea). The major important technologies that are now the foundation of the recent Coastal Fishing Ground Development Program (in Japanese "Engan Gyogyo Seibi Kaihatsu Keikaku") and sea-farming in general were developed through this program. These technologies include, but are not limited to the formation of mother scallop schools, the development of seaweed fields, the development of appropriate methods of releasing Kuruma prawns into tideland areas, and the acoustic conditioning of red seabream. The significance of predators was also recognized as one of the most important problems facing technologies enhancing propagation in the ocean environment.

Special Salmon Program: 1977–81

This program involved the large-scale production and systematic release of salmon fingerlings. Deployment was achieved in almost all of northern Japan, before the implementation of the U.S. and U.S.S.R. 200-mile zone policies. This program should also be recognized as a very significant effort in the history of world fishery resource development.

This challenging program resulted in the production and release of chum salmon (*Oncorhynchus keta*) fingerlings which reached 42.24 billion in number (equivalent to 140 thousand metric tons in total weight in 1987). It also contributed in educating many scientists, researchers, and technicians, bringing rapid technical improvements to many areas: fingerling release into rivers (Hokkaido); at-sea rearing and release of chum salmon (northeast Pacific coast); and technology to transport fish that grow up in other areas to the coast of the Sea of Japan.

There were also advances in the field of new species introduction, namely coho salmon (*O. kisutch*). This research has resulted in a large-scale tracking survey of juvenile and homing adult fish released in the coastal and offshore areas. We can say that this program has led to the recognition of new research topics, such as the importance of feeding and imprinting to breeding and fingerling production.

Marine Ranching Program: 1980–88

The marine ranching system is considered to be important not only for its contribution to research and development activities, but also as a public works project managed by the government. It has contributed many technical developments to a wide spectrum of marine aquaculture activities (i.e., offshore fish-cage development program). On the other hand, as a public works project, it has contributed the construction of large-scale propagation fields and artificial reefs under the Coastal Fishing Ground Development Program. Red seabream is a typical species shoes propagation has been successfully developed.

The Marine Ranching Program has been executed by integrating almost all of the research facilities from private, public, and academic institutions, such as the national and prefectural research laboratories, universities, and industrial research centers. In 1985, some thirty-six institutions were united together and committed to progress in this effort. It was also recognized that we would have to cope with many unknown and less-known fields to enable us to cross over to the use of an ocean ecosystem from our present use of conventional aquaculture and propagation systems. Based upon such recognition, The Marine Ranching Program has involved not only the fishery-related fields but also other biological, physical, and engineering sciences and technologies as well. This program consisted of five main categories which are divided into three phases according to its objectives and subsequent progress (Fig. 1). Phases I and II, conducted from 1980 to 1985, have already been finished with success after the expenditure of a total budget of 2.2 billion yen. During Phase III (1986–88), 1.2 billion yen was invested to initiate the Multiple-Resource Cultivation Systems, thereby combining and organizing the results of the previous phase's research. Each phase is outlined below.

Phase I (1980–82)

The total stock of a fishery resource depends upon mortality during the early stages of growth. During Phase I, ecological studies were conducted that highlighted this

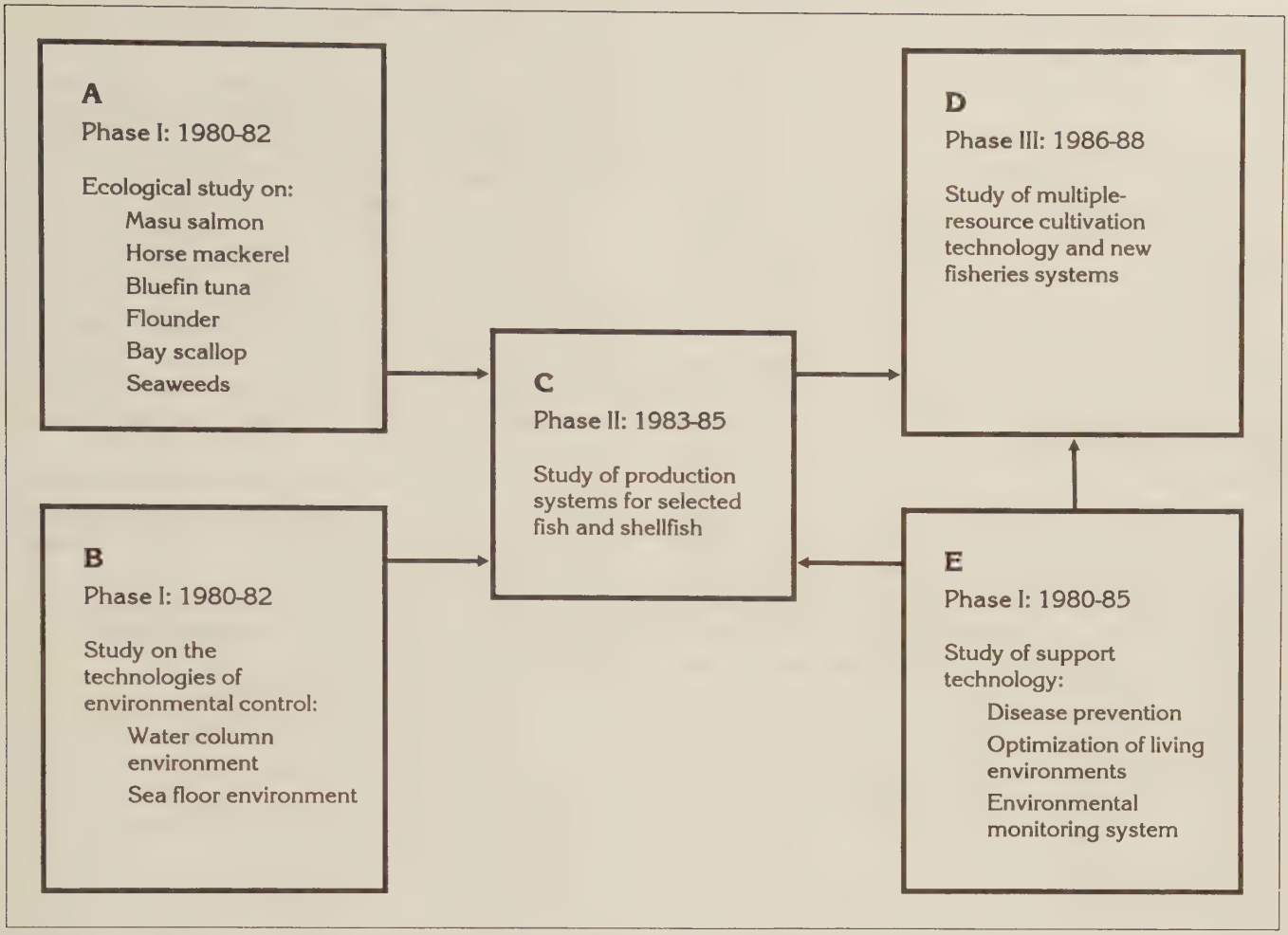

Figure 1
Relationship between the five catagories (**A–E**) of the Marine Ranching System, which were performed in three phases.

critical time for selected species. Other environmental studies on the control of water-flow and sea-bottom characteristics were also executed. The focal point of Phase I projects was to determine how, when, and where the best areas exist for us to approach the improvement of survival rates of fish during the juvenile stage of growth.

In order to organize information on groups of organisms sharing similar habitats and other characteristics, we categorized plant and animal species according to similarities in their life cycle. In some cases a single, selected species may represent an entire group for a certain category. Six groups were selected. They are as follows:

- Cherry salmon (*Oncorhynchus masou*)—fish which spawn after anadromous migration to rivers and estuaries;
- Japanese horse mackerel (*Trachurus japonicus*)—a species that spend its juvenile stage under the drifting seaweed;
- Bluefin tuna (*Thunnus thynnus*)—a species that migrates ocean-wide;
- Halibut (*Paralichthys olivaceus*), and flounder (*Limanda herzensteini*)—migratory bottom fish;
- Bay scallop (*Pecten albicans*) and ark shell (*Scapharca broughtonii*)—nonmigrating, sedentary fish which generally inhabit sandy bottoms;
- Sea oak (*Eisenia bicyclis*), sea trumpet (*Eclonia cava*), and gulfweed (*Sargassum fulvellum*)—large perennial algae which are located over rocky bottoms.

Phase II (1983–85)

The technologies proposed for the cultivation of the selected species resulting from Phase I research were examined at sea. As their effectiveness in controlling various aspects of the sea environment was measured, concurrent research on the structural and engineering technologies supporting those facilities, installations, and related equipment was also conducted. In addition, research was performed for optimizing other aspects of the ecosystem (i.e., indigenous

fish-life in the food chain) to address the area of feeding and other vital predatory or symbiotic factors for cogeneration with the important species.

Phase III (1986–88)

In order to effectively utilize the natural productivity of the sea, it is necessary to develop and construct suitable coexisting systems in both time and space dimensions among the multiple oceanic species while focusing on the selected important ones. We tried to develop a Multiple-Resource Cultivation System to upgrade the total productivity of the sea through the cultivation of some specific fish and shellfish. With this in mind, Phase III was started in 1986, based upon the results of Phase II research.

The multiple species selected included salmon, red seabream, abalone, and sea urchin—species for which cultivation technology had been developing. At the same time, the anticipated new fishery system for multiple resource cultivation was examined and integrated with the conventional technologies of propagation and aquaculture.

Conclusion

The research findings of the last phase are expected to provide the building blocks of fundamental technologies and basic knowledge to support several projects including 1) the Coastal Fishery Ground Development Program; 2) sea-farming programs; and 3) the "Marin-novation Plan." The Marin-novation Plan is currently being developed as a new integrated concept, combining fish cultivation with conventional fisheries as well as coastal area development (including design, deployment of port facilities, warehouses, and fish-processing plants). The plan is still only at a conceptual stage; no research and development programs or public works programs have been initiated yet.

We expect these research efforts to contribute technical developments in the cultivation of our fishery systems to support marine ranching into the 21st century. It is essential to realize that a close relationship and exchange of information must always be maintained among all the relevant scientists, researchers, and indeed the administrative staffs as well.

The Application of Current Techniques in Molecular Biology for Detection and Control of Infectious Diseases in Salmonid Aquaculture

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ABSTRACT

In recent years, significant progress has been made in the diagnosis and control of important human and animal diseases through the application of new techniques in molecular biology. These new techniques are being used to solve problems of salmonid aquaculture caused by those viral, bacterial, and deep tissue protozoan pathogens that do not respond to chemotherapy. Currently, losses from these diseases can only be prevented by efficient detection and avoidance of exposure to the pathogen. In the future, control of many fish diseases will rely upon the development of effective vaccines and genetically engineered molecules to prevent losses in geographic areas where pathogens are enzootic. This paper discusses the use of monoclonal antibody and DNA probe technologies to detect fish diseases and to create and test genetically engineered vaccines for enhancing the survival of Pacific salmon reared in aquaculture.

Introduction

Losses caused by infectious diseases remain one of the most important constraints to success in salmonid aquaculture. While some pathogens respond to drug or chemical control methods, many of the most significant diseases of salmon and trout are untreatable. Losses due to viruses, intracellular or drug-resistant bacteria, and some of the deep-tissue protozoans can only be prevented by avoiding exposure. This method relies upon the use of sensitive detection methods for the disease agents, the selection of disease-free animals, and the rearing of fish in pathogen-free water. The strategy is time consuming, expensive, and often impractical where pathogens are enzootic and the water supply cannot be treated. Recent advances in molecular biology have provided new tools for the rapid and sensitive detection of these diseases and for the development of genetically engineered vaccines for use in salmonid aquaculture. New detection methods, based upon the use of monoclonal antibodies and DNA probes, promise to be more rapid, sensitive, and cost-effective, while the genetic engineering of new types of vaccines and the creation of transgenic fish will allow aquaculturists to rear fish that are more resistant to enzootic pathogens.

Monoclonal Antibodies

Over a decade ago, Kohler and Milstein (1975) published a method for the creation of single clones of antibody-secreting mouse cells that could be propagated indefinitely in tissue culture and that would produce a single type of antibody. These "monoclonal" antibodies (MAbs) are superior to animal antisera for many applications because they are highly specific and very consistent. Several groups working with diseases of salmonid fish have used this technology to establish MAbs against antigens of infectious hematopoietic necrosis virus (IHNV) (Schultz et al. 1985; Ristow and Arnzen 1988; Winton et al. 1988), viral hemorrhagic septicemia virus (VHSV) (Enzmann et al. 1988; Lorenzen et al. 1988), infectious pancreatic necrosis virus (IPNV) (Wolski et al. 1986; Caswell-Reno et al. 1988), *Yersinia ruckeri* (Austin et al. 1986), *Aeromonas salmonicida* (Austin et al. 1986), *Renibacterium salmoninarum* (BKD) (Arakawa et al. 1987; Kaattari et al. 1987), *Vibrio anguillarum* (Goerlich 1987), *Vibrio salmonicida* (Espelid et al. 1988), and *Ceratomyxa shasta* (Hoffmaster et al. 1988).

These monoclonal antibodies have been used to distinguish among strains of IHNV (Ristow and Arnzen 1988; Winton et al. 1988), IPNV (Caswell-Reno et al. 1988),

BKD (Arakawa et al. 1987), and *Vibrio* sp. (Goerlich 1987) recovered from fish in different geographical areas and help provide a better understanding of the epizootiology and antigenic variation of the bacterial and viral isolates. Monoclonal antibodies have also become important reagents for the development of improved detection methods for fish pathogens (Austin et al. 1986). Because MAbs are very consistent and do not cross-react with other antigens to any significant extent, it is possible to design more sensitive assays allowing the detection of very low levels of antigen in infected fish. In addition, MAbs can be used to select or identify mutant strains of viruses or bacteria with altered properties (e.g. virulence) that will be effective as a vaccine against the wild-type isolates.

Recombinant DNA Technology

Among the most commonly performed operations of molecular biology are the cloning and sequencing of genes (Maniatis et al. 1982). In addition to providing fundamental knowledge about the structure and relation of genes and their products, this technology provides a powerful tool for detecting pathogens. By cloning either a DNA copy of the messenger RNA from a specific gene or fragments of DNA from the pathogen into a bacterial plasmid and expressing these in *E. coli*, it is possible to obtain a large amount of DNA with a nucleotide sequence that is homologous to a portion of the pathogen genome and that will bind strongly and specifically to it. Synthetic DNA can also be produced by automated chemical methods from an established sequence. This complimentary DNA (cDNA) can be labeled with radioactivity or an enzyme and used as a "probe" for the direct detection of very small amounts of the genetic material of the pathogen. Synthetic DNA "primers" have recently been used to amplify a specific sequence of DNA by a new technique known as the polymerase chain reaction (PCR) (Saiki et al. 1985, 1988). This method uses two primers and repeated rounds of polymerization to amplify nucleic acids with very low copy numbers to detectable limits (Guatelli et al. 1989). In addition to screening for genetic traits, this technique has been applied to detection of the human virus HIV-1 (Ou et al. 1988) and will soon be available for other animal diseases and genes. While still under active development, this technology promises to revolutionize fisheries genetics as well as the detection of fish diseases.

A second application of recombinant DNA technology is the development of new types of vaccines. "Subunit" vaccines can be made by inserting the gene for an important surface antigen of a pathogen into a bacterium which can then synthesize (express) high levels of the protein antigen as the bacterial cells multiply. Bacterial expression systems for production of the surface glycoprotein of IHNV and the major capsid protein of IPNV are under development at Oregon State University and workers in France and Belgium have collaborated to produce a subunit vaccine against VHSV.

Another novel type of vaccine produced by recombinant DNA technology is the insertion of pathogen gene sequences into a "viral vector." The ideal vector is a large DNA virus such as vaccinia, a poxvirus originally used to vaccinate humans against smallpox. This virus can accommodate large amounts of inserted DNA and animals can be infected with recombinant virus without producing disease. During replication of the avirulent recombinant, the inserted gene sequences are expressed, producing antigens which immunize the host. One problem with this approach for vaccination of fish is the current lack of a suitable vector. The only large DNA viruses of fish are either relatively pathogenic (iridoviruses) or are not widely distributed geographically (herpesviruses). If a suitable vector can be found, it is likely that this technology would be successful in fish.

Improved Detection of Salmonid Fish Pathogens

Serological tests using polyclonal rabbit (or other vertebrate) antisera have been widely used for detecting fish pathogens. One of the newer techniques, the enzyme-linked immunosorbent assay (ELISA) has been used as a rapid and sensitive test for *A. salmonicida* (Smith 1981; Austin et al. 1986; Adams 1988), *Y. ruckeri* (Austin et al. 1986), BKD (Dixon 1987; Pascho and Mulcahy 1987), IPNV (Nicholson and Caswell 1982; Dixon and Hill 1983; Hattori et al. 1984; Rodak et al. 1988), IHNV (Dixon and Hill 1984; Parkyn and Littlepage 1988), and VHSV (Dixon and Hill 1984).

A rapid ELISA-type assay where antigens are spotted onto filter paper and detected by labeled antisera (dot-blot) was reported by Sakai et al. (1987) for BKD and by McAllister and Schill (1986) for IHNV, VHSV, and IPNV. While these techniques initially used polyclonal rabbit antisera to detect the antigens, the development of monoclonal antibody-based ELISA and dot-blot assays will improve the precision of the tests.

Monoclonal antibodies have been used to detect IHNV antigen in cells and tissues of infected fish by immunofluorescence (LaPatra et al. 1988), immunohistochemical staining (Yamamoto et al. 1988), and by ELISA (Parkyn and Littlepage 1988; Arakawa, unpublished results) while Lorenzen et al. (1988) reported MAbs against VHSV were effective in detecting the virus by ELISA and immunofluorescence. Austin et al. (1986) used MAbs against *Y. ruckeri* and *A. salmonicida* to improve a novel "dipstick-ELISA."

Although fish have a less sophisticated immunological system than higher vertebrates, it is possible to detect

recent and past infection of fish based upon the presence of circulating antibodies. In addition to the traditional serum neutralization tests for viruses and agglutination or precipitation tests for particulate or soluble antigens, ELISA technology has been developed for detecting antibody in either vaccinated fish or fish recovering from infection (Bortz et al. 1984; Cossarini-Dunier 1985; Kodama et al. 1985; Hamilton et al. 1987; Thuvander et al. 1987). While caution should be used in the interpretation of ELISA data (Thorburn and Jansson 1988), this tool will find increasing use as a method for evaluating the immune state and past exposures of fish to pathogens.

Complimentary DNA probes for fish pathogens are now being evaluated as diagnostic reagents. Workers in the laboratory of Dr. Leong at Oregon State University have cloned the genes of IHNV (Kurath et al. 1985) and sequenced the gene coding for the glycoprotein (Koerner et al. 1987) and the nucleoprotein (Gilmore and Leong 1988). Recently, our laboratory has begun using a synthetic cDNA probe made from a portion of the published sequence of the IHNV nucleocapsid gene. We are testing this probe on IHNV infected cells and tissues to determine if the probe will allow the detection of very low levels of viral genomic or messenger RNA.

Vaccines Against Salmonid Fish Diseases

Currently, the only commercially licensed vaccines for use in salmonid aquaculture are preparations made from killed bacterial cultures (bacterins) of *V. anguillarum*, *V. ordalii*, *V. salmonicida*, *Y. ruckeri*, and *A. salmonicida*. These vaccines are generally effective, safe, and can be delivered by waterborne exposure.

Both killed and live-modified (attenuated) viral vaccines have been tested for protection of salmonid fish (Ellis 1988). While killed viral vaccines are safe and relatively effective, the high levels of antigen required and the apparent need to deliver the vaccines by injection make them expensive and somewhat impractical for use. Genetic engineering techniques have been used in an attempt to reduce some of these disadvantages by producing large amounts of pure antigen at lower cost.

Subunit vaccines consist of only the portion of a pathogen (usually a single protein antigen) that will stimulate protective immunity. While they can be prepared by extraction from large amounts of infectious material, the most efficient method involves cloning the gene for the major antigen into a rapidly growing bacterium (e.g., *E. coli*) and immunizing animals with a lysate of killed cells. These preparations are regarded as having a high level of safety. Because these bacterial expression systems are quite efficient, the cost of producing the antigen is relatively low.

One problem with subunit vaccines produced by cloned inserts in *E. coli* has been the inability of bacteria (procaryotes) to fold and process certain types of antigens in a completely normal way, making the synthesized protein unlike the native structure. Techniques to avoid this shortcoming involve the use of very short peptide vaccines that do not have to be folded and eucaryotic expression systems (yeasts, insects, and cell cultures) that can produce authentic antigens.

The development of recombinant virus vectors is another way to safely express high levels of authentic antigens. A large virus (e.g., vaccinia) can accomodate several gene inserts making polyvalent vaccines possible. This method of vaccination has the additional advantage of stimulating other forms of immunity (interferon, etc.) as the vector replicates. However, until the discovery of a suitable vector for the vaccination of fish, this method of protecting salmonid fish will remain theoretical.

Subunit vaccines for protection of fish against IHNV, VHSV, and IPNV are under development. The subunit preparation composed of a cloned insert of the transmembrane portion of the IHNV glycoprotein has been reported to confer protection to fish (Gilmore et al. 1988). A subunit vaccine against IPNV is under development (Leong et al. 1988). While some time and further experimental work will be required before these vaccines are fully tested and made optimal for protecting fish by waterborne delivery, it appears that subunit vaccines offer the best combination of safety and low cost.

Attenuated viral vaccines can be delivered by simple exposure and the weakened pathogen can be allowed to replicate in the animal, conferring an immunity that is often superior to that provided by killed vaccines. Because attenuated strains have the potential to revert to a virulent type or replicate in unwanted ways, the testing required for this type of preparation is usually extensive and the initial cost high.

Historically, attenuated viral vaccines were developed by serial passage of wild-type virus in cell cultures until the strain showed significant reduction in virulence. This required expensive testing. An attenuated IHNV strain was tested with encouraging results (Fryer et al. 1976), and de Kinkelin et al. (1980) described a thermoresistant variant of VHSV that could be used to protect rainbow trout. Recently, an attenuated strain of IHNV was developed by growing a wild-type virus in the presence of monoclonal antibodies against the virus, causing the selection of mutants with altered growth properties. Some of these mutants were also reduced in virulence and were able to protect rainbow trout against IHNV infection (Roberti 1988).

Future Developments

The techniques of molecular biology will continue to be

applied toward the solution of problems in salmonid aquaculture, resulting in greater production of healthy animals. One area in which significant interest has developed is the insertion of novel genes (or multiple copies of existing genes) into salmon and trout. While initial studies have focused upon the gene for the growth hormone, some workers have begun a search for the genes responsible for certain aspects of the fish immune system and the basis for the resistance of some species or stocks to specific diseases. These genes would be attractive candidates for creation of transgenic fish having improved survival in the presence of enzootic pathogens. Another possibility is the insertion of the genes for a more sophisticated immune system, perhaps from humans. While seemingly futuristic, the tools to perform these genetic manipulations are in hand (Mosier et al. 1988).

In addition to a key role in the development of subunit vaccines, molecular techniques are providing details of the nucleotide sequence responsible for key receptor sites on the surface of cells or pathogens. These are believed to serve as recognition and attachment points for complimentary sites on some viruses and bacteria. The engineering of specific molecules to block these sites is being explored as a control method for acquired immune deficiency syndrome in humans. This technology may one day be applied to fish as well.

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Physical Considerations for the Design of Algal Drift Traps

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ABSTRACT

Commercially important algal herbivores, such as abalones and sea urchins, feed mainly on detached pieces of macrophytic algae called algal drifts, and rarely on the attached forms. Artificial structures built to trap these drifts have been incorporated in projects seeking to develop abalone and sea urchin propagation grounds in Japan. Only recently, however, have scientific studies on the trap design been conducted. This paper is one of the first contributions with this approach. Two hydraulic characteristics of algae were investigated. First, specific gravity was observed to vary by season, age, and species. The rough ranges of specific gravities in seawater of *Laminaria* spp. and *Eisenia bicyclis* were 1/40∼1/20 and 1/25∼1/10, respectively. Second, it was found that the friction drag coefficient of *L. religiosa* fronds was increased considerably by their corrugated shapes. The trapping effects of the block-type trap in a two-dimensional vertical steady flow were studied with scale model experiments. It was revealed that there exists an optimum trap height for maximizing the trapping efficiency of the block. A method is presented which provides a rough estimate of the trapping efficiency when the block height is greater than or equal to the optimum value.

Introduction

Large brown algae of the order Laminariales are an important food of abalones and sea urchins, two commercially important species of nearshore rocky areas. In these wave-exposed areas most of the biomass produced by these algae is estimated to be unavailable to the algivores for the following reasons. Abalones and sea urchins feed mainly on detached pieces of attaching macrophytes called algal drifts, but rarely on the attached forms (Olsen 1968; Shepherd 1973; Dayton 1985; Imai and Kodama 1986). The detached algae are so easily moved by water motion that most of them are swept out of the habitats.

In Japan, both field researchers and planners of government-funded projects building abalone or sea urchin propagation grounds have been trying to trap algal drifts with various types of structures. However, the projects have relied on intuition, and their field surveys have focused solely on quantifying the amount of trapped algal drifts or examining the change of the resulting herbivore biomass. Without giving greater attention to physical considerations, one cannot hope to design the best possible trap.

A few scientific papers concerning the design of algal drift traps have appeared recently; this paper deals with the development of systematic approaches to the complicated and vague problem of how to maximize and estimate the effects of the algal drift traps. First, the physical characteristics of detached algal fronds, a little known area, are discussed. Based on the results of experiments using artificial and natural fronds under controlled conditions, important physical indices were developed to estimate the motion of algal drifts. Second, owing to the variety of conceivable structural configurations which are possible, the algal drift traps have been broadly classified into two types, block and screen. The former type is an impermeable structure formed by close-packed concrete blocks or natural stones and is commonly used for the construction of aquaculture grounds. Its trapping effects in relation to a two-dimensional vertical steady flow are described in detail in the text. A more detailed discussion of the screen type, mentioned only briefly here, can be found in Kawamata (1987).

Hydraulic Characteristics of Algal Drifts

Algal drifts are flat, floppy, and ruffled fronds released from the substrate, with a density only slightly greater than

Table 1
Specific gravity of attached *Laminaria* collected off Taneichi, Iwate, 1987.

Date	Species	Age (yr)	No. of samples	Specific gravity (average ± SD)
11 Mar.	*L. religiosa*	1	9	1.063 ± 0.003
10 Jul.	*L. japonica*	1	10	1.052 ± 0.003
11 Mar.	*L. religiosa*	2	17	1.068 ± 0.007
10 Jul.	*L. religiosa*	2	10	1.076 ± 0.004

Table 2
Specific gravity of drifting *Eisenia bicyclis* collected off Choshi, Chiba.

Date	No. of samples	Specific gravity (average ± SD)
14 Apr. 1987	5	1.066 ± 0.011
21 Aug. 1987	4	1.128 ± 0.038
03 Mar. 1988	9	1.080 ± 0.008
12 Nov. 1988	10	1.088 ± 0.016

seawater, which makes them fairly lightweight in seawater. Owing to the increased surface area of their corrugated bodies, they experience a greater drag force than a flat plate of the same dimensions. That is the reason why detached algae may be easily carried away, even by a slow current, or may be raised from the sea bottom by turbulence in rough seas.

Forces governing the behavior of an algal drift are the drag imposed by the fluid, buoyancy-reduced gravity, and the elastic force against bending. Elasticity is one of the important factors to which attention should be paid when making models of the algal drifts. For instance, a flexible model with only a little corrugation can be gradually turned up from its edge, even by a relatively slow current, while a stiff one cannot. Most algal drifts may be sufficiently flexible to be considered non-elastic bodies. Thus model algal drifts used experimentally should be made of flexible materials which deform well in moving water.

The hydraulic characteristics of algal drifts have not been sufficiently investigated yet. In order to improve on this lack of information, the author presents the results of sample surveys and experimentation using natural fronds.

Specific Gravity

Tables 1 and 2 show the specific gravities of attached *Laminaria* spp. and drifting *Eisenia bicyclis*, which were collected near the shores of Taneichi, Iwate, and Choshi, Chiba in different seasons. The specific gravities of the algal fronds s_a were measured through the following formula:

$$s_a = s \frac{W}{W - \Delta W}, \quad (1)$$

where s is the specific gravity of the seawater, W is the weight of the frond in air, ΔW is the weight of the frond in seawater, which was measured by underwater balance.

On one occasion, 10 July 1987 (Table 1), cultivated *L. japonica* were collected in place of wild one-year-old *L. religiosa*, which could not be found at the sampling of *Laminaria* off Taneichi. In the coastal area *L. religiosa* is binennial but most of the individuals disappear in a year and *L. japonica* is cultivated with culture lines in the sea (K. Chiba, Iwate Prefectural Northern Sea Farming Center, Taneichi, Kunohe, Iwate, 039-13, pers. commun., Nov. 1989). Taxonomically, however, both species closely resemble each other in regard to both the morphology and distribution (Miyabe 1902). Yabu (1964) proposed to treat *L. religiosa* as varieties of *L. japonica*. Kawashima (1989) mentioned that *L. religiosa* was originally derived from biennial *Laminaria* such as *L. japonica* and turned to an annual under severe environmental conditions such as high water temperature or shortage of nutrients as it is distributed into the warm current regions. He also stated that *L. religiosa* could turn back to a biennial according to environmental conditions. *L. japonica* is thus morphologically similar to *L. religiosa*, and both species can be regarded as being almost equal in specific gravity to each other. Specific gravities of *L. japonica* are close to the values of one-year-old *L. religiosa* early in summer; these gravities have also been measured by Kawamata and Hagino (1987).

The density of *Laminaria* fronds tended to increase with aging except during the first summer after becoming sporophytes. This trend seems to be connected to mucus concentrations since most parts of the body with higher proportion of mucus had a smaller density (Kawamata and Hagino 1987). Mucus is a photosynthetic product. Therefore the idea that mucus has a smaller density because of its higher water content, agrees with the fact that the density of one-year-old *Laminaria* fronds is minimized early in summer when their rapid growth, which began in the winter, ceases (Abe et al. 1985). The increase in frond density with aging after this point might be due to an increase in the ratio of tissue which is greater in density and which stiffens the frond.

Both the density of the *E. bicyclis* frond and its seasonal change were greater than the *Laminaria* spp. *E. bicyclis* is a perennial. The seasonal variation in density of *E. bicyclis* probably depends on the seasonal variation in the dry-weight content of the frond. According to Asakawa et al. (1988), the dry-weight content of *E. bicyclis* vaired withseason, high (30%) in summer and fall, and low (10%) in winter.

For the purpose of this study, the specific gravity of seawater is about 1.025; therefore, the specific gravities in seawater of *Laminaria* and *Eisenia* fronds can be regarded as about 1/40 ~ 1/20 and about 1/25 ~ 1/10, respectively.

Friction Drag Coefficient

The drag exerted on an algal drift depends on its changeable shape, and the incident angle and velocity of the current. The following are experimental results from Toda (1983) and Kawamata and Hagino (1987) who measured the friction drag exerted on the *L. religiosa* fronds shown in Table 3 flapping in uniform flow, whose stipes were tied with string to a spring balance. In these cases, the friction drag coefficient C_f is defined by the following formula (Schlichting 1968):

$$C_f = \frac{1}{2}\frac{Dg}{\rho u^2 A \times 2}, \qquad (2)$$

where D is the total friction drag exerted on the frond as measured by the spring balance, g the gravitational acceleration, ρ the density of the fluid, u the current velocity, and A the blade area. Figure 1 illustrates the friction drag coefficients against the Reynolds number $R_l = ul/\nu$, where l is the blade length and ν kinematic viscosity of the fluid. Hino and Utahara (1977) measured the friction drag which acted 1- or 2-cm wide, 1-m long vinyl films flapping in a uniform flow. They obtained a greater friction drag coefficient than that of a smooth, flat plate at zero incidence, as shown in Figure 1. However, the friction drag coefficients of the *L. religiosa* fronds were even larger than those of the vinyl films. This indicates that the corrugated shapes of algal drifts are also one of the important factors governing the motion.

It should be noted that the methods of Toda and those used by Kawamata and Hagino were slightly different. Toda used a frond tied with a single string that allowed the frond to rotate around the string. Kawamata and Hagino put a frond on a thin steel bar, which was fixed parallel to the direction of the current, by tying it loosely at several points to the flume wall with strings in order to inhibit rotation. The friction drag coefficients obtained by Toda were a little greater than those obtained by Kawamata and Hagino as a result of these differences in methodology.

Table 3
Laminaria religiosa fronds used for the friction drag test.

Investigator	No.	Blade length (cm)	Mean blade width (cm)	Blade area (cm^3)
Toda (1983)	P1	320	6.74	2157
	P2	122	9.27	1131
	P3	220	7.40	1628
	P4	380	8.41	3196
Kawamata and Hagino (1987)	P5	244	6.89	1681
	P6	221	7.08	1565
	P7	148	4.38	648
	P8	264	7.29	1925

Two Typical Types of Algal Drift Traps

In general, algal drift traps may be divided into the following two types:

- The block type, which traps algal drifts on the front and back sides by controlling the surrounding fluid motion.
- The screen type, which entangles drifting algae with little variation of the flow.

The block-type trap produces turbulence, including flow separation mentioned later. Quite a few studies are available on the velocity profile and the turbulence characteristics in the vicinity of a block (Pande et al. 1980; Nakagawa and Nezu 1987). However, the information provided by these studies is far from sufficient for the purposes of this study, because the behaviors of algal drifts are related to both the stochastic phenomena in the turbulent flow and the fine structure of the algal drift. In this paper, the relationship between the hydrodynamic processes and behaviors of algal drifts are experimentally described from an engineering viewpoint.

As previously stated, the trapping effect of the screen-type trap has been experimentally explored and discussed in detail by Kawamata (1987). In a steady flow, the screen is very effective in trapping algal drifts and the trapping effect varies little with current velocity. In an oscillatory flow induced by waves, however, the screen is far less effective, because algal drifts entangled with the screen are dislodged by the oscillatory flow at once. Eubenthic animals are thus unlikely to capture the moving drifts.

Block-Type Traps in Two-Dimensional Vertical Steady Flow

In order to explain simply the important flow patterns and behaviors of algal drifts in a two-dimensional vertical flow, let us consider a thin rectangular block held on a flat bed in a free-surface steady flow as shown in Figure 2. The flow around the block is characterized by two separation bubbles. The first is found on the upstream side of the block, where the boundary shear layer on the bottom first separates at a point upstream of the block and then re-

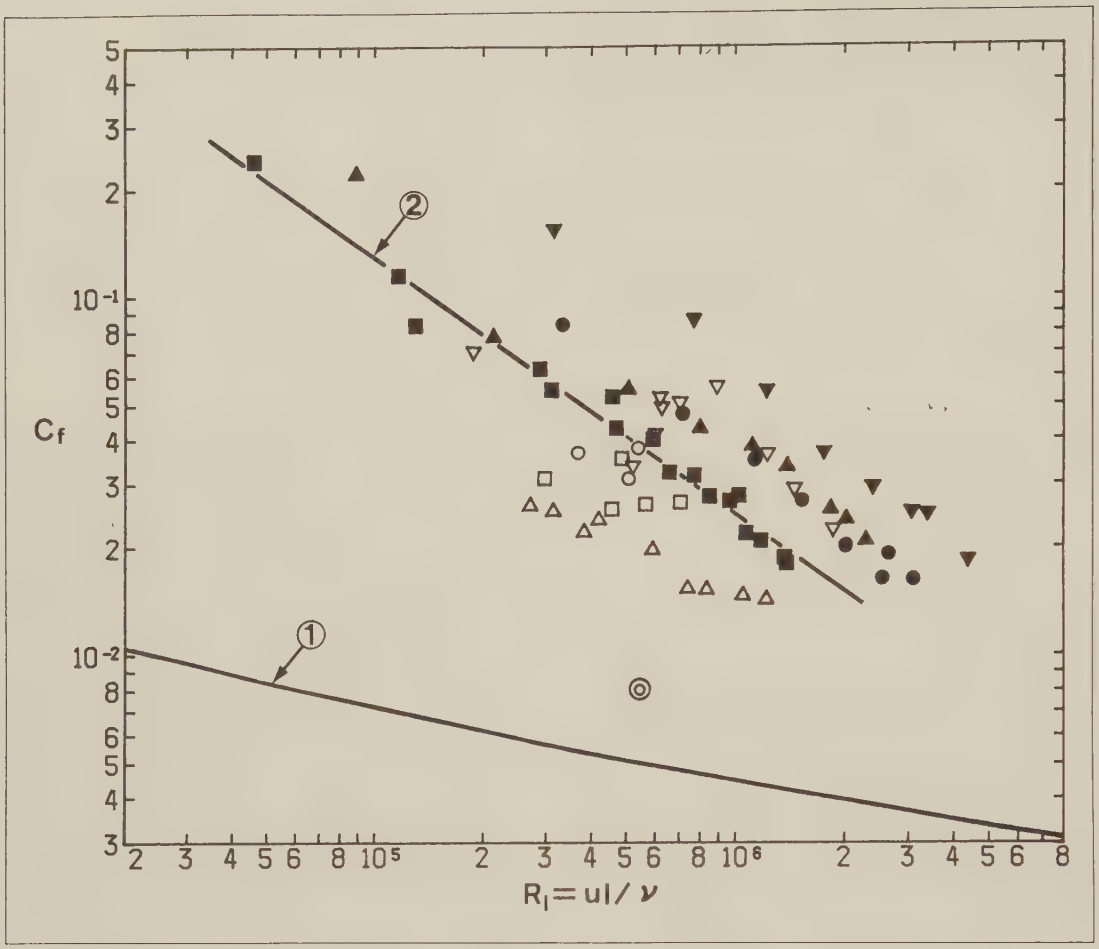

Figure 1

Friction drag coefficient C_f versus Reynolds number R_l; comparison of *Laminaria religiosa* fronds and other flat objects. u = current velocity, l = length of an object, ν = kinematic viscosity of fluid. Each mark in the graph represents the frond described in Table 3: ● = P1, ■ = P2, ▲ = P3, ▼ = P4, ○ = P5, □ = P6, △ = P7, ▽ = P8. ◎ = average of the friction drag coefficients of vinyl films (Hino and Utahara 1977); ① = friction drag coefficient of smooth flat plate at zero incidence (Schlichting 1968), ② = regression line of the frond of P2, $C_f = 503\ R_l^{-0.718}$.

attaches to the boundary. The downstream separation bubble is created by the flow passing over the block which separates on the top of the block and reattaches to the bottom. These separated flows which reattach to the boundary at a downstream point give rise to recirculation regions with reversed flow.

When an algal drift is released on the upstream side of the block, there are four distinguishable phases in its behavior which are dependent on current velocity. In the first phase there is a state of no movement because the current is too slow. In the second phase a drift is transported to and captured near the separation point ahead of the block. In the third phase it passes over the block and falls into or is trapped by the downstream separation bubble with a certain probability according to the flow velocity. In this case it is moved back by the reversed flow where it stays immediately behind the block. In the presence of high flow rates there is no probability of its staying near the block and thus we see the last phase. At the thresholds of each phase, there are three characteristic velocities, the "critical velocity for movement," the "critical jumping velocity," and the "critical non-stay velocity." These processes have not been quantified yet but may be schematically summarized as in Figure 3. As Postoma (1967) mentioned, critical values in sediment transport depend on current velocity only indirectly; the important factors are the tractive forces acting on the bottom, such as the roughness of the bottom and turbulence. However, because this paper does not aim at hydrodynamic calculations, it is sufficient to use flow velocity.

Trapping Efficiency of the Block

Because algal drifts seem to be trapped most frequently

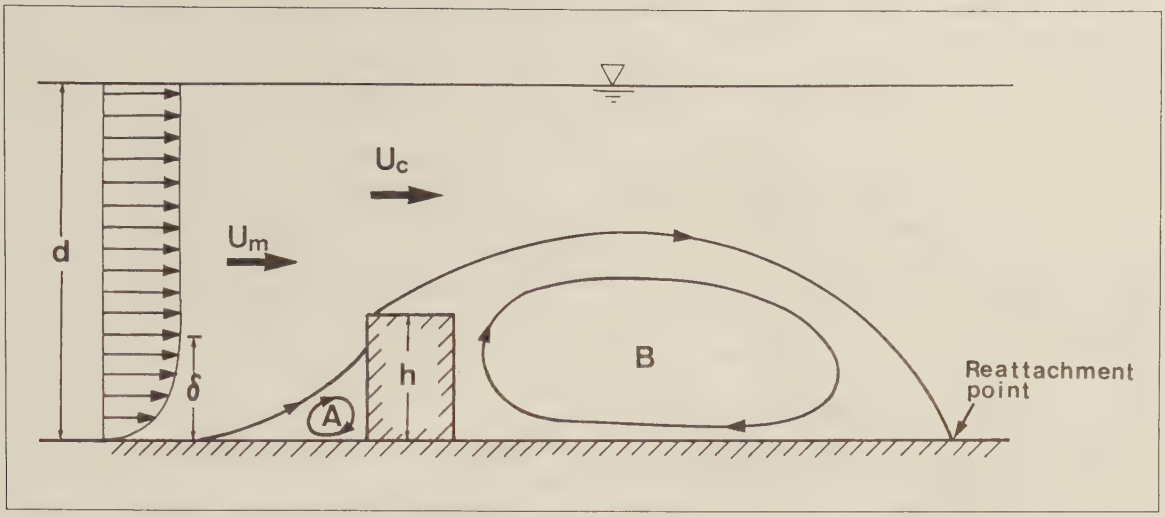

Figure 2

Schematic diagram of the free surface steady flow past a submerged block. A = upstream separation bubble; B = downstream separation bubble; d = water depth; h = block height, δ = boundary layer thickness; U_m = mean bulk flow velocity in the uniform flow section; $U_c = U_m\, d/(d-h)$ expresses mean bulk velocity of the flow contracted by the block.

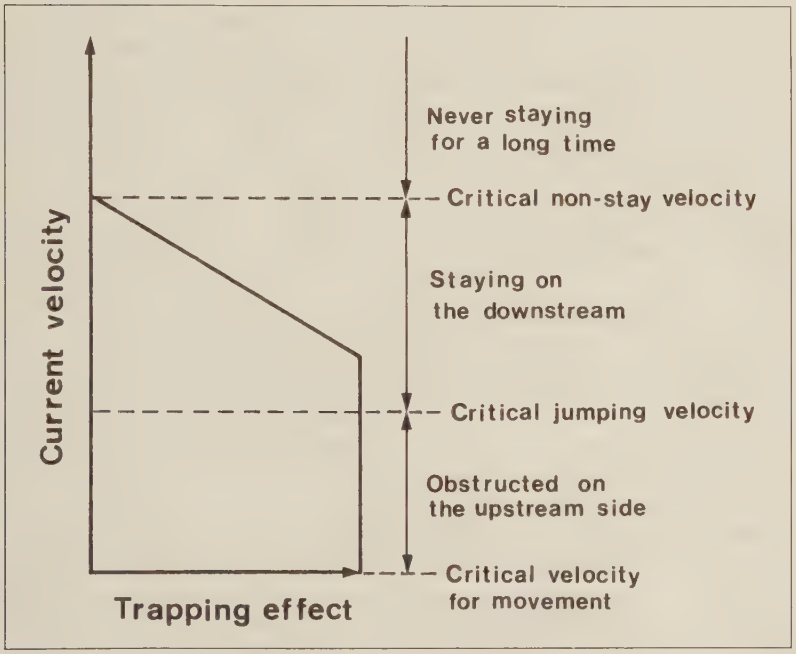

Figure 3

Schematic diagram of variation of the trapping effect against current velocity.

on the downstream side of an obstruction in nature, the author has investigated the trapping effect in the third phase in detail. Reduced scale hydraulic experiments were performed which studied the behavior of model algal drifts.

Materials and Methods—Experiments approximating a one tenth scale were conducted in a horizontal flume, 39-m long, 40-cm wide, and either 30-cm or 40-cm deep. A test section was set up for the release of model drifts by first covering the channel bed over a length of 360 cm with acrylic plastic boards in the middle of the flume. A 5-mm thick plate-shaped block of height h (2 cm to 15 cm) and width to span the entire channel was fixed to the bottom in the middle of the test section. Subcritical uniform flow conditions were set up in which the variation of the free surface by the block was very small. The Frode numbers $F_r = U_m/\sqrt{gd}$ were 0.04 to 0.07 and the Reynolds numbers $R_e = U_m d/\nu$ were 25500 to 48800, where U_m is the mean bulk velocity in the uniform flow section, and d the water depth. Considering the hydraulic characteristics of the real

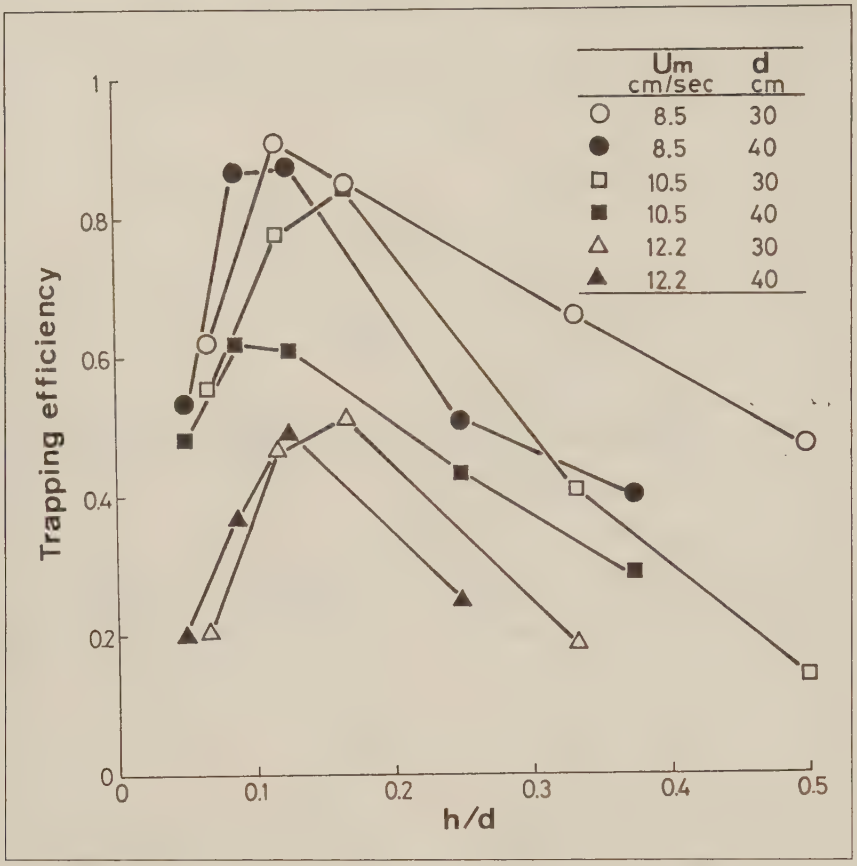

Figure 4
Trapping efficiency versus dimensionless block height (h/d). U_m = mean bulk flow velocity in the uniform flow section; d = water depth.

algal drifts mentioned above, the first model drifts were made of boiled *Undaria pinnatifida* blades, whose dimensions were 9 mm × 100 mm. The model drifts were released into the flow about 180 cm upstream from the block with a tool that made them start moving on the bottom with a minimum of turbulence. Most of the released model drifts soon passed over the block by passing the second phase. The model drifts passed the block stochastically, staying for a long time in the third phase, having been trapped by the downstream separation bubble. The trapping efficiency of the block was defined as the probability that a drift of particular hydraulic characteristic, should be trapped permanently behind it. A minimum of three replicates of 15 model drifts each were released at one time. About five minutes after they reached the block, both the number of the drifts passing over the block and the number staying behind it were counted for each experiment. Fifteen models were thought to be about the maximum number which could be liberated at one time without becoming entangled; an observation time of five minutes was considered sufficient for the drifts to be stabilized.

The trapping efficiency for each experimental condition was calculated as follows:

$$\text{Trapping efficiency} = \frac{\text{Total number of drifts staying behind the block}}{\text{Total number of drifts passing over the block.}} \quad (3)$$

Results—The observed values of the trapping efficiencies were plotted against the dimensionless block height h/d in Figure 4. The trapping efficiency increased with height below a certain value. However, when the block height exceeded this value the trapping efficiency decreased. This was unexpected because the length of the downstream separation bubble increases approximately in proportion to the height (Pande et al. 1980). The variation of the trapping efficiency against the block height can be explained by the following two effects. The first positive effect is the protection of an algal drift which stays behind the block from the turbulence. For example, the downstream separation bubble produced by a higher block in the turbulent boundary layer prevents a larger vortex coming over the block from carrying away algal drifts caught within it. Therefore, the block height should be greater than a certain value in order to protect algal drifts from being removed

downstream. On the other hand, a second negative effect of height occurs, which destroys the boundary layer and contracts the flow. The original flow velocity at the level closer to the bottom is slower in the boundary layer. A higher block increases the velocity of the flow on the downstream separation bubble, which forces the fluid inside it to recirculate. At the same time, especially in shallow water, a higher block also increases the velocity by constricting the cross-section area of the flow. These make the shear stress between the main stream and separation bubble greater, and consequently the recirculation flow, which acts on a drift behind the block forcing it to rise out of the reversed-flow region, faster.

The reattachment point is, on average, where an object of the same density as the fluid in which it is transported from the separation point should settle on the downstream bottom. Therefore an algal drift past the block should tend to fall down on the bottom a little upstream of the reattachment point because of the action of its underwater weight. The underwater weight also acts to prevent an algal drift located closely behind the block from being raised up. The effects of the underwater weight on the motion of the algal drift diminishes relative to drag force when the current velocity increases. Thus, when the block is much higher than the boundary layer thickness, the trapping efficiency is reduced. The interaction of the two positive and negative effects establish the optimum block height value to trap algal drifts on the downstream side. The optimum height might be the minimum one at which the vortices shed in the area upstream from the block do not reach the bottom immediately behind the block through the downstream separation bubble. The value may depend on the boundary layer thickness or the upstream turbulence, both of which this study does not examine. The following part of this paper is limited to the range of the block height greater than the optimum value.

Indicator of Trapping Efficiency

Figure 5 shows the conversion of Figure 4 into the relationship between the mean bulk velocity of the flow contracted by the block, $U_c = U_m d/(d - h)$, and trapping efficiency. The mean bulk velocity, U_c, explains the traping efficiency quite well except for the lowest height. This suggests that the drag force, which governs the motion of the algal drift, should be represented in a particular situation. Here the drag exerted on an algal drift flapping in a steady flow of velocity U_c, denoted by D_c, was measured as the representative value of the drag. The relative magnitude of D_c and the weight of the drift in water, ΔW, would indicate the trapping efficiency. This supposition was experimentally verified.

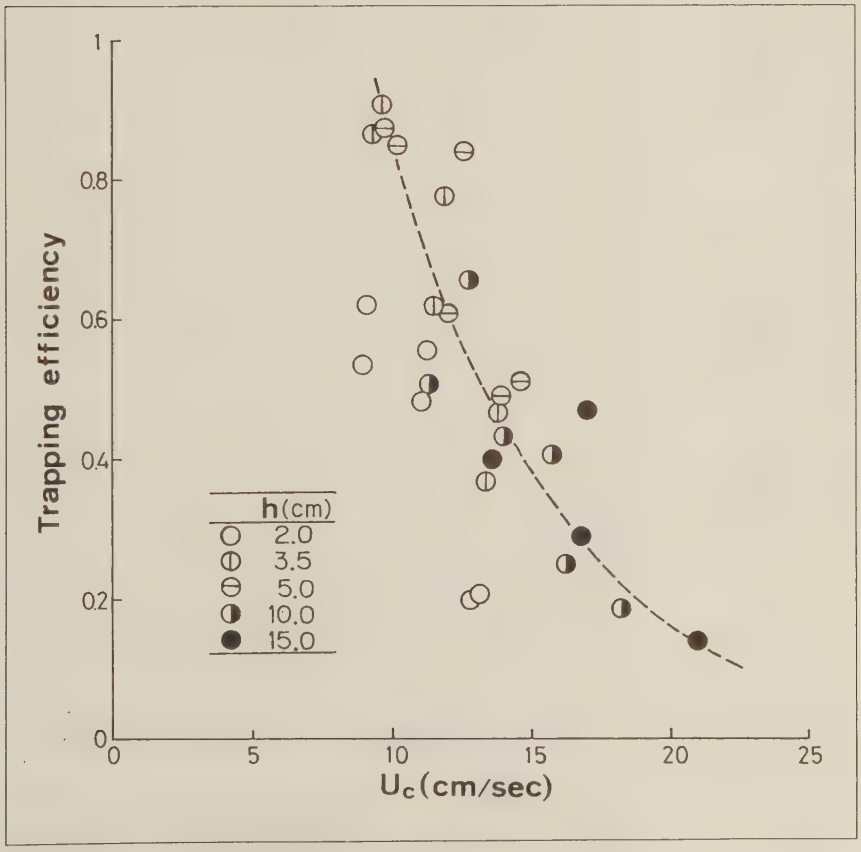

Figure 5
Relationship between trapping efficiency and U_c (mean bulk velocity of the flow contracted by the block) obtained by the rearrangement of Figure 4.

Table 4
Scale models of algal drifts and associated hydraulic conditions.

No.	Dimension (mm)	Material of scale model	ΔW[a] (dyne)	Drag[b] (dyne)	Hydraulic conditions[c] U_m (cm/sec)	d (cm)	h (cm)
M1	9 × 100	Boiled blade of *Undaria pinnatifida*	6.4	$1.21u^{1.36}$	12.2	30	5
M2	10 × 100	Nylon textile	1.6	$0.83u^{1.66}$	12.2	30	5
M3	10 × 100	Nylon textile	2.7	$0.83u^{1.66}$	12.2	30	5
M4	10 × 100	Nylong textile	5.5	$0.83u^{1.66}$	12.2	30	5
M5	10 × 100	Polyester textile	3.7	$0.83u^{1.66}$	12.2	30	5
M6	10 × 100	Polyester textile	5.6	$0.83u^{1.66}$	12.2	30	5
M7	10 × 100	Polyester textile	8.2	$0.83u^{1.66}$	12.2	30	5
M8	10 × 100	Celophane film	8.8	$2.85u^{1.38}$	12.2	30	5
M9	20 × 200	Boiled blade of *Undaria pinnatifida*	28.7	$18.8u^{0.95}$	17.7	60	10
M10	18 × 200	Cellulose acetate film	38.2	$21.8u^{1.08}$	17.7	60	10

[a] ΔW = weight of drift in water.
[b] u = current velocity (cm/sec).
[c] U_m = mean bulk velocity over the block; d = water depth; h = block height.

Materials and Methods—Ten sorts of model drifts were made using *U. pinnatifida* blades and man-made materials including nylon, polyester, cellophane, and cellulose acetate. Releasing experiments were conducted as previously described. Table 4 indicates the physical properties of the models (M1–M10) and hydraulic conditions under which the drag values were determined. Models M8 and M10 were adequately ruffled by hand because their original bodies were so flat that they often remained glued to the bottom.

Results—Figure 6 shows experimental results. Most of the plotted values approximated the broken line (estimated by hand) with the exception of models M1 and M9 which were both made of boiled *U. pinnatifida* blades. These models had lower trapping efficiencies, probably because the viscosity resistances acting on their smooth peripheries to raise them up from the bottom were greater than on the other models with rough textures.

It was indicated, however, that the ratio $D_c/\Delta W$ might be used to get a rough estimate of the trapping efficiency in actual fields.

Discussion

Information is still insufficient to indicate fully the proper practical design for this type of algal drift trap. However, it is easy to estimate the effects of the algal drift trap with existing information. Presented below are some useful suggestions for designing a block-type trap long enough to be governed by the principles involved in a two-dimensional vertical flow.

- The "optimum value" is probably the best height unless the trap is permeable. Algal drifts are likely to be trapped on the upstream side of a high trap with a high efficiency, but in practice, algal drifts transported toward the trap stop a certain distance upstream of it, where they are easily moved sideways even by slow transverse currents. Therefore the trap height should be the minimum value for the retention of the algal drifts behind the trap, that is, the optimum value, so as to make them jump over the trap and be caught behind it easily. Moreover, special attention should be paid to the fact that the optimal height of a permeable trap such as the type developed by Kawamata (1988) is greater than that of an impermeable one.
- The trap should be set up in as uniform a flow as possible: In turbulent flows, oncoming vortices over the bottom may raise algal drifts behind the trap. In wave-induced oscillatory flows, vortices are alternately generated on both sides of the trap without the creation of recirculation regions; therefore, algal drifts are unlikely to stay around the trap. This also indicates that the trap should be placed on as flat a bottom as possible and that other structures ought not be located either parallel to or near it. This is because artificial obstacles, as well as rugged sea bottom, break the benthic boundary layer and induces vortices.

Considering these actors, traps are probably less effective in shallow water near the shore because stronger

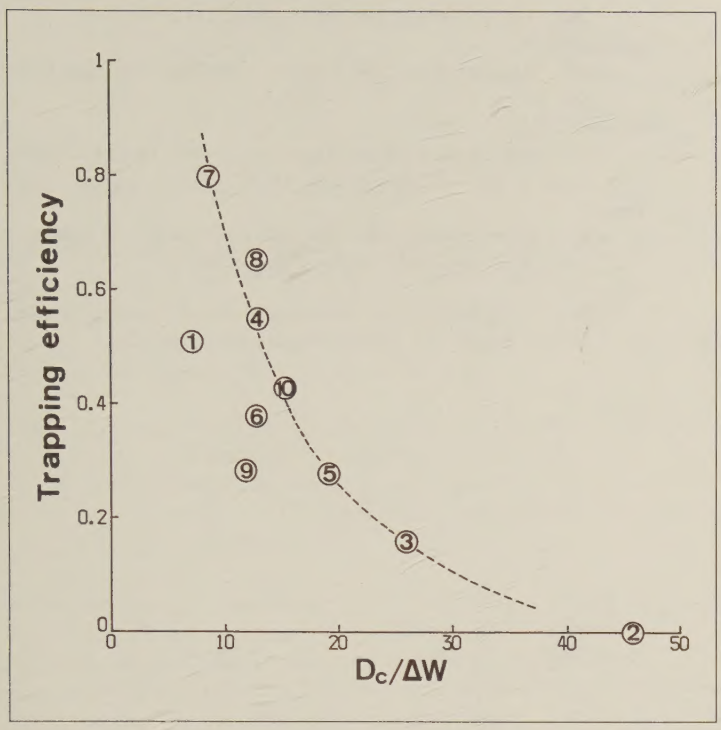

Figure 6
Relation between the trapping efficiency and $D_c/\Delta W$. D_c = drag exerted on an algal drift flapping in a steady flow of velocity U_c; ΔW = weight of an algal drift in water; numbers in ○ = nos. of the models described in Table 4.

wave action and rugged bottoms made by erosion do not produce uniform fluid conditions. On the contrary, in deeper water the oscillatory flow induced by waves weakens and near-steady flows, such as tidal and ocean currents, prevail. In addition, the seabed in these regions is generally flatter owing to sedimentation of sand and absence of scouring. Thus, from the physical viewpoint, traps will have a greater effect in deeper water than in shallower zones.

Moreover, distribution of algal vegetation is in general different from that of the algivores. Macrophytic algae dominate on hard substrates close to shore and are less abundant in deeper water, while algal herbivores, especially sea urchins, tend to live in deeper water away from the zone of dense macrophyte forest (Dayton 1985; Kawamata and Hagino 1986). Such an ecological phenomenon also supports the proposal that traps should be set up in deep, barren grounds to supply hungry herbivores with algal drifts.

In conclusion, the trapping efficiency of the block-type trap is probably estimated by the current velocity and the friction drag coefficients of algal drifts, as described in Figure 1, and also by the relationship between the trapping efficiency and $D_c/\Delta W$ shown in Figure 6.

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